ALGAE IDENTIFICATION AND ENUMERATION COURSE

lecture and laboratory notes compiled for the instruction of water works operators

Ontario

Ministry of the Environment

The Honourable George A. Kerr, Q.C., Minister

K.H. Sharpe, Deputy Minister

ALGAE
IDENTIFICATION
AND
ENUMERATION
COURSE

LECTURE AND LABORATORY NOTES
COMPILED FOR THE INSTRUCTION OF
WATER WORKS OPERATORS

WATER RESOURCES BRANCH
LIMNOLOGY & TOXICITY SECTION

3RD EDITION
REVISED MARCH, 1970

ALGAE IDENTIFICATION AND ENUMERATION COURSE

Day and Time	Subject	Section No.
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11:00-11:20	Identification of Plankton (General)	2
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1:15-3:00	Laboratory: Use of Microscope Identification of Animal Plankton and Invertebrates	4
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ALGAE AND OTHER INTERFERENCE ORGANISMS IN WATER SUPPLIES

WHAT ARE ALGAE?

Algae are plants just as trees and grass are plants. They are green, they manufacture their food in the form of starches or oils by using the energy of sunlight and the nutrients they extract from the water. In the classification of plants, they are considered to be the most primitive groups, and some of the algal forms commonly found in water supplies are thought to be similar to the first life on earth. They are considered primitive because each cell is capable of carrying out the complete life history as no specialization has been developed into various tissues such as are found in the higher plants (stems, roots, leaves, seeds). All higher plants and animals are composed of millions of cells. Throughout the ages, certain cells of plants and animals have arranged themselves into specific organs (kidney, heart, liver, skin) which carry out a highly specialized function. In algae, each individual cell fulfils all such functions, i.e. excretion, respiration and reproduction.

VALUE OF ALGAE

The water works operator often looks upon algae as purely a nuisance as it clogs filters, imparts tastes and odours to the water supply, and causes growths on reservoir walls.

Algae are however, the basis of all life in water. On land, grass feeds the rabbit which in turn is eaten by the fox and while the fox does not eat grass, there would be no foxes if there were no grass. This is called the food chain and the basis of the food chain in water, is algae. Algae feed minute animals which in turn are eaten by minnows which in turn provide the food for pickerel. Thus, if there were no algae there would be no pickerel. It can be domonstrated that fish production in a lake varies directly with the amount of algae that it produces and thus while it may be a disadvantage in a water supply, it is not unnatural and it is a necessity for other uses we make of water. When the first men start making long distance trips into space, the food they will consume will be algae grown in the space capsule.

SIZE AND DISTRIBUTION

There are several thousand different species of algae that live in the waters of Ontario. These range in sizes from a plant as much as four feet tall down to cells which are so small that they can barely be seen when magnified a thousand times in a microscope. In addition to living in the oceans, lakes and rivers, down to the depth where the light can penetrate, they also live on the damp soil on the face of glaciers, and in combination with fungi to produce the lichens we are all familiar with.

GROWTH REQUIREMENTS

Algae are very specific in their needs. The types that are characteristic of lakes are seldom found in streams and those which populate a lake in summer give way to other forms in winter. Some species can only live in very pure water and others are obligated to polluted situations and even sometimes to particular types of pollution. Factors governing the type and number of algae are environmental such as temperature, available light, and nutrient concentrations such as nitrates, phosphates, manganese, iron etc. No two species of algae have exactly the same requirements.

SIGNIFICANCE AND INTERPRETATION OF ALGAE

Algae are normal and constant inhabitants of nearly all natural surface waters. Wherever algae grow, there too will be found bacteria, fungi, and various animals. These different organisms interact in the open waters to carry on a chain of life. The algae as green plants use the dissolved solids (nutrient minerals), water, and carbon dioxide to grow and reproduce. Bacteria and animals feed on the dead or live organic algae. The bacteria in turn die, and their bodies are broken down, thus eventually returning the elements to the mineral condition. If any part of this cycle were to be eliminated or altered, the resulting water might contain materials that would cause tastes and odours, or be otherwise undesireable as a water supply. The living creatures serve to stabilize the water and to degrade or decompose foreign material, such as pollution, that may enter the aquatic environment.

The more frequently observations on a raw water source are made, the greater the likelihood of noting the beginning of increased algal populations. Counts may vary at a few hundred Areal Standard Units per ml. for an extended period, then climb perceptibly to a few thousand A.S.U. per ml. within three to four days. Such increases may result from growth stimulated by a change in the weather; by an increase of nutrients from sewage effluents, land drainage, precipitation, or applied fertilizers; or from planktonic masses drifting out from adjacent fertile tributaries.

Excessive numbers of one algal type may cause no end of trouble in the water treatment plants. It is therefore important for the plant operator to know what is in the water. No specific rules can be set down which will account for all local circumstances and it would be misleading to quote a number of plankton counts and imply that the consequences of each would always be the same.

Water works operators can learn a great deal about the general quality of the water by an examination of the phyto-plankton populations. Certain generalizations can be made:

(a) Severely polluted, warm or hard waters tend to encourage blue-green algal forms.

(b) Cold, clear waters generally favour diatoms.

(c) Green algae tend to be abundant during spring and late fall seasons.

Thus, one of the great benefits that can result from a systematic program of phytoplankton counting is to predict expected "blooms".

ALGAE PROBLEMS IN THE WATER SUPPLY SYSTEM

The operator can be faced with problems created by algae of several kinds. In all cases, they result from an over-abundance, but the numbers required to create this difficulty will vary.

1. Filter Clogging

The reduction of filter runs, caused by the coating on the surface of the filters with large numbers of these minute plants is probably the most common and serious problem that algae create for the water works operator. Certain waters at certain times of the year produce a great abundance of the filter clogging species and under the worst conditions may reduce the production of water through a filter to a point where there is hardly sufficient water to backwash. The lake diatoms such as Asterionella, Melosira, Synedra, Tabellaria, Fragilaria and Stephanodiscus are the most common trouble makers in this regard, but certain of the summer blue-green algal forms may also develop in sufficient numbers to reduce filter runs. This problem has occurred at water works plants along Lake Ontario where Melosira has caused a reduction in the normal filter run.

2. Taste and Odour Production

Algae are capable of producing tastes and odours that will persist through treatment and cause consumer complaints. Bacteriological doses of chlorine often rupture the algal cell wall thus expelling the cell's waste material into the water supply system. Certain blue-green algae such as Anabaena, Aphanizomenon and Microcystis are well known for developing very foul "pigpen" odours in water. These blue-green forms collect in large masses sufficient to form water "blooms". The foul odour undoubtedly develops from products of decomposition as the algae begin to die off in large numbers.

Generally, different algae have been shown to cause different flavours and odours that have been variously described as pigpen, grassy, musty, cucumber, etc. Much of the difficulty of tastes and odours is the result of the decomposition products of the rooted aquatic weeds. Raw water supplies that come from

shallow, weedy lakes almost invariably have a continuous or intermittent problem with tastes and odours. This problem is usually most acute late in the fall when the ice-cover has just formed and again in the spring at the time of the break-up of ice-cover. Clarke Lake near Bancroft recently had the odour-producing flagellate Synura present in its waters. This algae in low numbers imparted a perceptible cucumber-like odour to the water.

3. Growths in Reservoirs

A third common problem that algae create in a water works operation is growths in reservoirs. Here the algae may grow attached to the walls where they form a heavy mass of material. This algal mass may be alive with crustaceans and insect larvae. Occasionally, one of these little animals may come through the taps and shake the confidence of the consumer in the purity of the supply. Algal growths in the reservoir may be of a free-floating type. These growths may impart tastes to the water. Probably the most common algae causing difficulty in reservoirs is one of the larger species called Chara. This algae grows to a height of two or three feet in a soft mud bottom and is typical of the cold, hard water commonly found in spring water sources. Where such water is collected and stored in an open reservoir this algae invariably grows and is difficult to control.

CONTROLLING ALGAE

There are two basic means of controlling algae and solving the problems that they create; one, by controlling the environment in such a way as to make it an unsuitable place for them to live, and two, by treatment practices. The latter method is less satisfactory in the long run as it necessitates adding chemicals that are costly on either a continuous or intermittent basis. Controlling the environment is a more satisfactory means, though this is not always possible.

1. Environmental Control

The best method of this type of control is by selecting the best possible source of supply. A deep, cold lake rather than a shallow, warm productive lake should be chosen. In choosing a new supply care should be taken to utilize water of low fertility as judged by chemical analyses and the algae population that it maintains. Routine biological and chemical samples should be obtained for at least one year previous to the installation of any water works facilities. Limnologists would then be able to determine the numbers and kinds of algae present and assess the suitability of the water. Where the municipality controls the land adjacent to the supply, care should be taken to keep out surface drainage and other possible nutrient sources. Run-off from buildings, domestic sewage and certain

industrial wastes are rich in plant nutrients and should be avoided as only small amounts of these fertilizing substances can induce the development of high algae populations.

Another aspect of environmental control is to exclude the light by covering pre and post treatment reservoirs. The easiest method is simply to cover the reservoir, although this is often not done and algae problems continue year after year. This may be achieved by a permanent cover such as concrete or a black plastic sheet spread over the reservoir. While the latter method has not been used, it would be effective in excluding light and be relatively inexpensive to apply. A second method of reducing light in pre-treatment reservoirs is to use activated carbon to induce an artificial turbidity. While this is only a temporary measure and must be repeated every four days, it has the added advantage of adsorbing taste and odours from water while in suspension and keeping the bottom accumulation sweet. Carbon can only be used in the raw water where the treatment following includes good sound filtration.

2. Treatment Practices

(a) Control of Filter-Clogging Algae

The obvious way to solve the problem of short filter runs is to remove the algae before they get to the filter. The common method of removing algae from raw water is the use of settling basins which may follow flocculation and pre-chlorination. Microstrainers are also being used to remove the algae before the water is filtered. A third method is to apply algicides to the raw water and thus remove them before the water enters the plant.

The principle of removing algae by flocculation and sedimentation involves trapping the algal cell in the alum floc and carrying it to the bottom with other unwanted solids from the water. When algae populations are very high they often hold the floc in suspension long enough for it to pass through the settling basins and onto the filters. This floc and the algae can be settled if weight can be added to the floc. A slurry of ordinary clay mixed and fed during the periods of difficult times will do much to get the operator over a short term period of difficulty. Increased dosages of alum and heavier pre-chlorination will also assist in alleviating short filter runs.

(b) Chemical Control

While there are many algicides sold today, only two are suitable for use in a domestic water supply, namely copper sulphate and chlorine. The cheapness and availability of copper sulphate and its safety from a public health standpoint make it the most satisfactory chemical to use. The effectiveness of copper sulphate varies somewhat with the chemical composition of the water. In hard water the copper precipitates rapidly thus, more is required than in soft water. As a rule of thumb .25 ppm will kill most algal forms although a dosage of .5 ppm is required for some forms. Copper sulphate is very toxic to fish and about 1 ppm is about all they will stand. Copper sulphate is sold in a variety of crystal sizes. The method of application varies with the grade of crystal used. In general, the finest crystals may be distributed on the surface of the water as they will immediately dissolve. Larger sized crystals may be dissolved in water and pumped as a spray or they may be put in a burlap bag and towed through the water in such a way as to provide an even distribution of the calculated amount of the chemical over the entire surface of the reservoir. The operator must know the depth of water as he applies the chemical and sees that the deeper water gets proportionally more chemical than the shallow areas.

In applying chlorine, a rough calculation must be made of the volume of water being treated and the pounds of chlorine required to satisfy the demand and still provide a residual of 1 ppm. The calculation is used as an initial guide, then followed by chlorine tests to provide the final adjustments. A similar calculation must be made for determining the amount of copper sulphate but more care must be exercised as no simple test can be used as a guide. To do this, the surface area of the water to be treated must be obtained together with the average depth of the water. When multiplied these two figures give the volume of water in cubic feet. The total number of pounds of water may then be calculated by multiplying the volume by 62.5. As one part per million (ppm) equals 1 pound per million pounds of water, the treatment of a reservoir with .5 ppm. would require one half pound for each million pounds of water.

Area x average depth x 62.5 = lbs. of water in reservoir

1 ppm = 1 lb. per million lbs. of water

The ideal time to apply chemicals is when the algae population is rising but before the condition becomes acute. If treatment is postponed until a very dense growth of algae occurs the sudden killing of this material and the subsequent decomposition may remove all the oxygen from the water causing it to go septic, kill the fish, and become foul-tasting. If the condition gets out of hand before treatment can be applied, half the reservoir should be treated first to reduce the population; after a week or so has been allowed for this material to decompose, the total reservoir area can then be treated.

(c) Microstraining

Microstraining as a method of water treatment was introduced in Ontario about 9 or 10 years ago. There are about 6 installations operating on municipal water supplies. The development of this means of filtration was made possible by the invention of an extremely fine wire mesh capable of removing such small particles as algae from the water and yet capable of passing high volumes of water. The principle of the microstrainer is simply a rotary screen where the raw water is fed to the inside and flows out through the screen material. The drum is about three quarters immersed and as it turns around, a jet of water is played on the surface of the screen and knocks down the accumulated solids and algae into a hopper and from there are carried to waste.

In water treatment the microstrainer has two uses:

(a) as pre-treatment for algae removal ahead of conventional sand filters,
(b) as sole treatment for waters for the removal of algae and other extraneous material where turbidity is not a problem.

Microstrainers have proven very effective in extending the operating time of conventional sand filters during times of heavy algae "blooms". In one instance, runs of not less than 20 hours have been obtained where previously 6-hour runs in summer were not uncommon and as little as two hours were experienced. Where a microstrainer is used as a sole means of water treatment it should never be installed with the thought of reducing turbidity. Where it has been used solely for the removal of algae and the protection against the variety of water fleas, insect larvae, leeches and aquatic worms, that commonly pass through unprotected water supplies, it has been found to be very satisfactory.

OPERATION OF MICROSTRAINERS

Some of the microstrainers installed in the province have been set up on an automatic control system and some are operated manually. The system used will depend on the individual plant. In general, they are easy to operate and require the normal lubrication and an occasional wash down. Over a period of time some permanent plugging of the screens will take place that is not backwashed by the water jet. When this occurs, the strainer must be drawn down and a 12% sodium hypochlorite solution applied directly to the fabric while the screen turns over slowly. It should be emphasized that concentrated chlorine solutions from a chlorinator or from chlorine powders are not effective in rehabilitating the screen capacity.

The reason for the sliming of the fabric is not well understood. The time between washing has varied anywhere between one day and six months and in one or two instances difficulties due to lessening of filter capacity over a period of one or two days have occurred. In all cases, the screens have been quickly rehabilitated with the hypochlorite wash and an investigation is now underway to obtain a continuous method of protection against this short term loss of capacity.

(d) Control of Algae-Caused Tastes and Odours

Two methods are commonly used in controlling tastes and odours; one, the feeding of activated carbon and two, variations in the method of chlorination. Activated carbon is the only sure method of taste removal, but this on a continuous basis is somewhat expensive. Also, this method is somewhat messy. Another method of controlling tastes and odours is to alter the method of chlorination. Super-chlorination or break-point chlorination may be helpful. These methods must be tested as the algae may be killed leaving the decomposition products to make conditions worse. The use of chlorine dioxide or chloramine at various points of application in the plant may assist in controlling tastes and odours but this is an individual problem within each water works and can only be determined through experimentation.

WATER MAIN INFESTATIONS

While water main infestations do not necessarily come under the title of this section, there has been considerable interest in this matter in recent years, and so perhaps a little of the fact and fiction should be separated. It is probable that most if not all water distribution systems contain living organisms of some kind. A brief survey of the literature indicates the wide variety of animals that have caused difficulty from time to time. These have included nematodes, aquatic earth worms, snails, clams, a variety of aquatic insect larvae, leeches,

Gordian or horse hair worms, Daphnia or water fleas, etc. Many of these have occurred in municipal supplies having complete treatment of flocculation and sand filtration. The method of entering the distribution system often remains a mystery though in many cases it is thought that the minute egg passes through the sand bed and develops subsequently in the distribution system. This problem is not more widespread because the inside of a water main is relatively clean so that food is available only in very limited quantities. In some cases the life cycle cannot be completed entirely under water so that insects such as blood worms cannot reproduce in the water main.

NEMATODES OR ROUNDWORMS

These are very small animals barely visible to the human eye. Some authorities consider them to be the most numerous animals on the earth as they are found in the soil, on lake bottoms, along the shores, in sewage sludge and in fact in almost any sample of earth. In view of their great numbers, it is not surprising that a few of them find their way into water distribution systems. Here they are able to subsist on the thin coating of slimes lining the water main and in organic depositions in low flow sections of the distribution system.

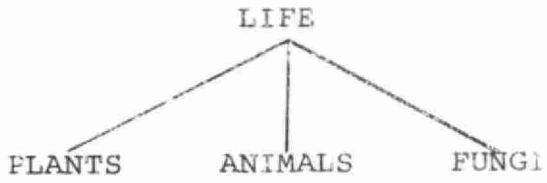
In this country, Nematodes have no public health significance; they carry no diseases, and are not human parasites. Although it is not likely known, many Nematodes are consumed by an individual every year in raw fruits, salads, vinegar and perhaps even in his drinking water. They are quite resistant to chlorination and the levels normally applied for the control of pathogenic bacteria are not sufficient to kill Nematodes. In this respect, we are fortunate as this climate controls some species that are a serious human problem in the Middle East and Asia.

CONTROL OF INFESTATIONS

As most of the organisms that inhabit water mains are resistant to normal water works sterilization procedures and as no chemicals are suitable for adding to water to control this type of nuisance organism, good housekeeping is the only effective control. As much as possible of the organic material should be kept from the water mains. This is best accomplished through chemical precipitation and sand filtration. In laying water mains, low flow areas should be eliminated as much as possible, as they provide a refuge. Where dead ends occur in the system, they should be flushed routinely. In this way, it will be possible to maintain the confidence of the consumer in the products that you deliver.

NOTES

IDENTIFICATION OF PLANKTON (GENERAL)



Plants

Plants are photosynthetic, i.e. in the presence of sunlight they can synthesize inorganic nutritional materials to provide for growth and reproduction. This is made possible by the presence of the pigment chlorophyll which imparts the green colour to all plants, large and small. While the proper classification of some of the single-celled forms of life is open to dispute among microbiologists because some have both plant-like and animal-like characteristics, a simple means of differentiation is to categorize all organisms which contain chlorophyll as plants. Sometimes other pigments are present in algae which impart a blue-green or brown colour to certain forms of these tiny plants because the chlorophyll is masked by these other pigments. (e.g. blue-green algae - phycocyanin).

PHOTOSYNTHESIS

$$\text{Carbon Dioxide} + \text{Water} \xrightarrow[\text{and chlorophyll}]{\text{in the presence of sunlight}} \text{Starches \& Sugars} + \text{Oxygen}$$

This is an extremely simplified formula indicating what takes place as plants manufacture their food. A balanced condition is provided by the fact that animals breathe the oxygen produced by plants in order to metabolize their food and release energy for movement and other bodily activities, at the same time producing carbon dioxide which is essential to the plants.

Fungi

Fungi are a somewhat specialized group which for a long time have been classed by most biologists as plants. However, they are distinctly different in that they do not possess chlorophyll and are unable to synthesize their own food. Fungi are able to secrete enzymes which change insoluble food to a soluble form which is assimilated and metabolized within the cells.

Animals

Most animals ingest and break down solid food of organic origin although there are some unicellular forms which assimilate soluble food materials through their cell membranes. They cannot produce their own food and never contain chlorophyll.

Planktonic forms of interest to us represent the Plant and Animal Kingdoms. Algae are the tiny plants, mostly microscopic in size, that are extremely influential in affecting water supplies in certain areas. In addition, there are both unicellular and multicellular animals which are encountered and which may cause nuisances also.

Algae

These tiny, chlorophyll-bearing plants may be single-celled or the cells may be grouped together in filaments or colonies.

Although there are quite a number of major groups of algae which are recognized by taxonomists, for purposes of simplification, the algae which are significant in water supplies may be grouped into four different types. These are as follows:

1. Blue-green algae
2. Non-motile green algae
3. Pigmented flagellates
4. Diatoms

The Classification of Organisms

One of the first questions usually posed about an organism seen for the first time is: "What is it?" usually meaning, "What is its name?" The naming or classification of biological organisms is a science in itself (taxonomy).

The system of biological nomenclature is regulated by an international congress. It is based on a system of groups and super groups, of which the foundation is the species. The categories employed are as follows:

The species is the foundation.

Similar species are grouped into genera (singular: genus).

Similar genera are grouped into families.

Similar families are grouped into orders.

Similar orders are grouped into classes.

Similar classes are grouped into phyla (singular: phylum).

Similar phyla are grouped into kingdoms.

The scientific name of an organism is its generic name plus its species name. This is analogous to our system of surnames (family names) and given names (Christian names). The first letter of the generic (genus) name is always capitalized and that of the species name is written with a

small letter. Both names should be underlined or printed in italics when used in a technical sense.

i.e. Homo sapiens - modern man.

Esox lucius - northern pike.

A complete list of the various categories to which an organism belongs is known as its "classification". This may be written as follows for Phacus pyrum, a green flagellate:

Kingdom	-	Plantae
Phylum	-	Euglenophyta
Class	-	Euglenophyceae
Order	-	Euglenales
Family	-	Euglenaceae
Genus	-	Phacus
Species	-	pyrum

Algae which are found in water supplies are placed in four general groups as discussed in sections 5 to 13. The proper taxonomic Orders of algae found in fresh waters are as follows:

1. Bacillariophyceae
2. Chlorophyceae
3. Chrysophyceae
4. Cryptophyceae
5. Dinophyceae
6. Euglenophyceae
7. Myxophyceae (Cyanophyceae)

See Appendix A for a more detailed discussion on the taxonomic classification of algae.

NOTES

ANIMAL LIFE ENCOUNTERED IN WATER SUPPLIES

Unicellular forms of animal life are collectively referred to as Protozoa. Five major groups of Protozoa are recognized as follows:

1. Sarcodina - e.g. Amoeba, Arcella
2. Mastigophora - e.g. Bodo
3. Ciliata - e.g. Paramecium, Vorticella
4. Sporozoa - not of interest - occur as parasites in plants and animals
5. Suctoria - e.g. Acineta

Representatives of the Sarcodina groups have pseudopodia, finger-like processes which develop and are constantly changing in shape and the animal literally "flows" as it moves along very slowly. Pseudopodia may also be extended to engulf food particles which are assimilated through the cell membrane of the animal into the protoplasm within the cell. Protoplasm is the jelly-like constituent of all plant and animal cells in which all of the basic life processes occur. The Mastigophora group is comprised of those forms which bear flagella (singular - flagellum). These are whip-like appendages which are used in movement. Flagellates move in cork-screw-like fashion. The possession of cilia is the outstanding characteristic of the Ciliata. Cilia are hair-like appendages which cover the body of the cell or are located at the anterior end of the animal. They are used in movement and in some cases for capturing food. Both free-swimming (Paramecium) and attached (Vorticella) forms of ciliates are often encountered. The Suctoria possess tentacles which are used to sting other organisms which pass nearby and to suck the contents from their victims.

Multicellular Animals

Rotifers

These more complex animals have one or two crowns of cilia at the anterior end of the animal which resemble wheels. These cilia beat to permit movement and to create suction currents for drawing in food particles. The mastax is a powerful set of jaws which is clearly evident in the body of the animal which grinds up food with a hammer-like action.

AQUATIC WORMS

Small aquatic "sludgeworms" are related to terrestrial earthworms. These tiny worms are able to withstand low dissolved oxygen conditions and are frequently observed as pink or red carpets on the bottoms of polluted streams. They are occasionally observed on the filter beds in water filtration plants.

Midge Larvae or Blood Worms

These segmented insect larvae are often vivid red in colour and are wormlike in character. They may be distinguished from the aquatic nematodes by the fact that they are somewhat thicker in diameter and microscopic examination may reveal hooks and breathing tubes.

Daphnia and Cyclops

These are tiny relatives of the common crayfish which feed on algae and are an important food item for small fishes. They appear as tiny white specks which move through the water with a jerky motion.

Scuds or Amphipods

These are somewhat larger crustaceans than the Daphnia and Cyclops, which may grow up to half an inch in length. They move smoothly through the water and their abdominal gills may be seen functioning when the animal is at rest.

Hydras

These tiny fresh-water coelenterates have a columnar body usually 15 to 20 mm. in length with a ring of tentacles around one end of the body, which are used to paralyze and capture prey. Hydras have been known to live on the walls of filter beds and to sometimes clog filters when they reach particularly high numbers in lake waters.

Moss Animals or Bryozoans

Various treatment plants along Lake Ontario and Lake Erie have reported the presence of free-floating statoblasts of Pectinatella. The statoblasts are reproductive bodies from which larger jelly-like colonies develop. Other bryozoans form mosslike brownish mats which are attached to the bottoms of lakes or streams.

Terminology Used in the Description of Protozoa

Pellicle - the cell membrane which determines the shape of the animal and encloses the inner components of the cell.

Protoplasm - the jelly-like substance which is contained by the pellicle in which the animal's life processes function. The protoplasm is divided into a clear outer portion called the ectoplasm and a granular inner mass, the endoplasm.

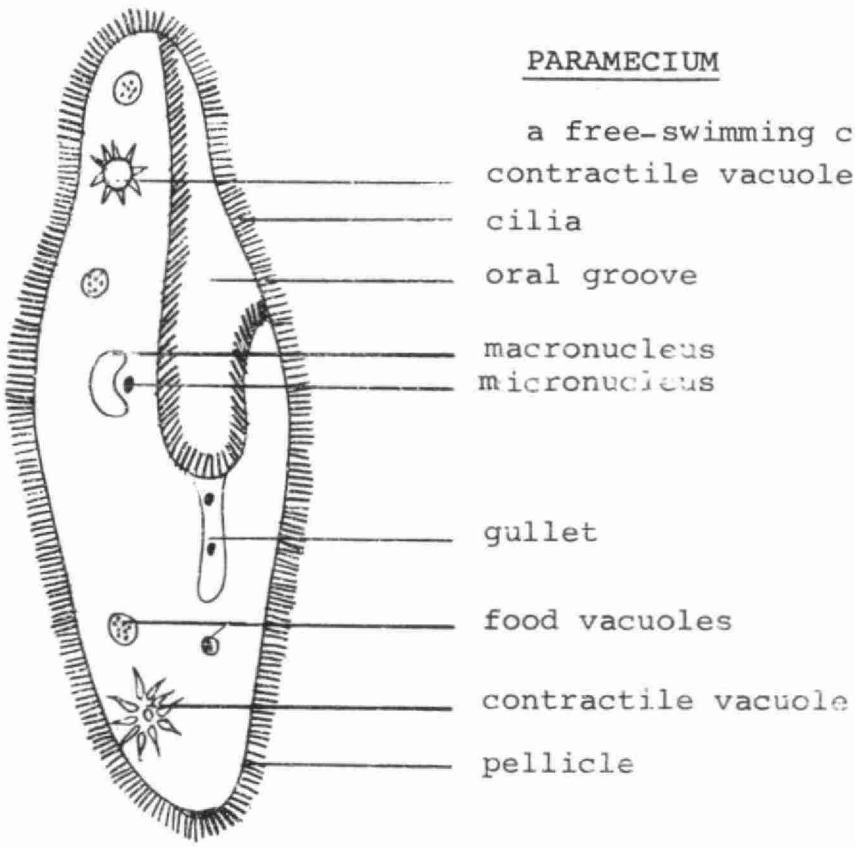
Macronucleus - the larger nucleus which governs bodily activities within the cell. Not present in Sarcodina.

Micronucleus - the smaller nucleus which is involved in reproduction.

Contractile Vacuole - a relatively large, clear structure which is responsible for gathering and excreting water from the cell.

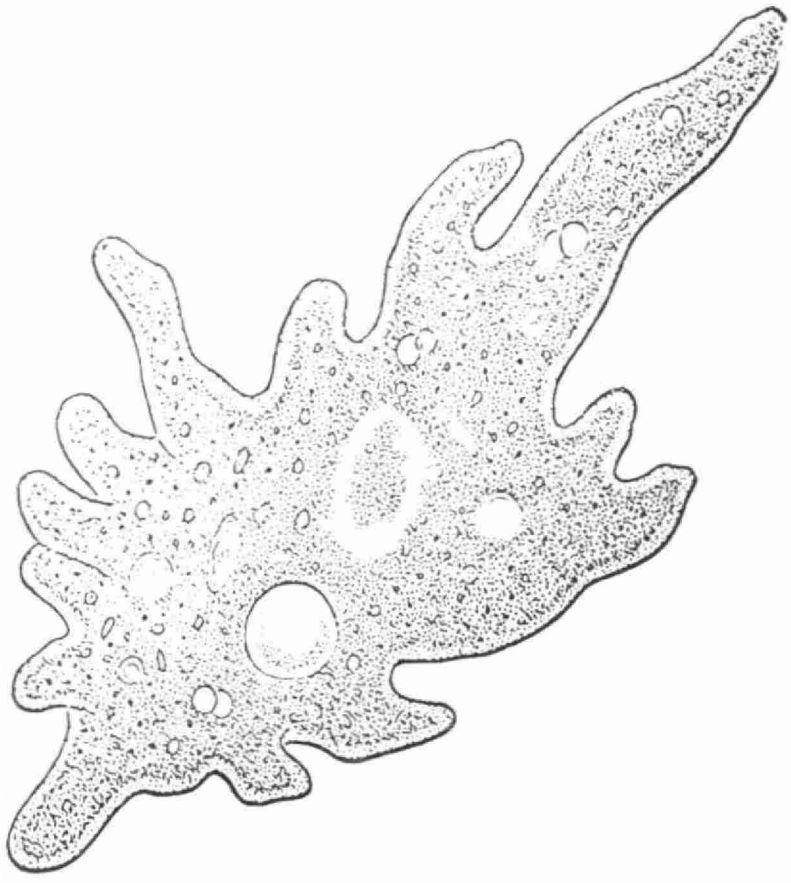
Food Vacuoles - structures in which food is being broken down by enzymic action.

Oral Groove - present in ciliates - opening lined with cilia into which food praticles are drawn.

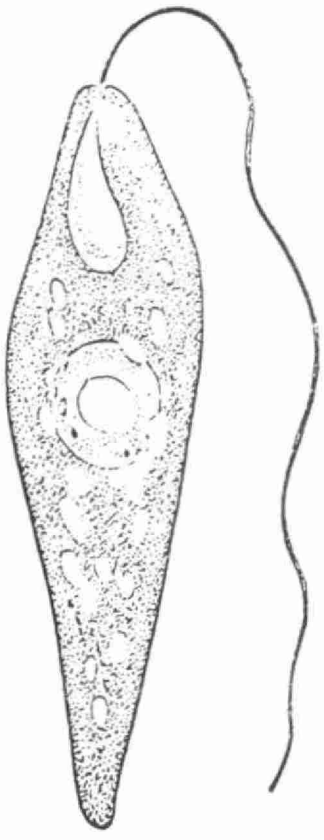


PARAMECIUM

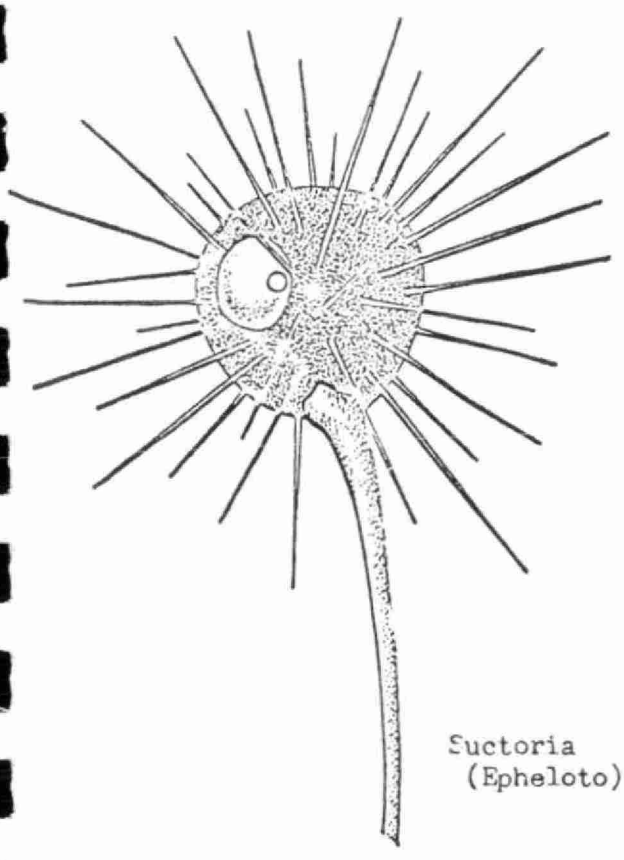
a free-swimming ciliate



Sarcodina
(Amoeba)



Mastigophora
(Euglena)



Suctoria
(Epheloto)

Ciliata
Vorticella)

NOTES

LABORATORY - FAMILIARIZATION WITH MICROSCOPE AND LEARNING TO RECOGNIZE ANIMAL PLANKTON

Objectives

1. For beginners to learn how to use microscope.
2. To learn to identify animal life encountered in plankton counting and to associate these with basic animal types.

A. Use of the Microscope

1. Note the major parts - objectives, eyepiece, stage, light source, coarse adjustment knob, fine adjustment knob, co-axial handle to position slide.
2. The bottom left-hand corner of the Sedgwick-Rafter cell is centered under the objective. Note how the image appears to the eye - upside down, and on the opposite side.
3. Practice focusing with the objective fixed over the edge of the Sedgwick-Rafter cell to gain an idea of the depth of field at 100X.
4. The magnification achieved is the product of the power of the eyepiece and of the objective. A 10X eyepiece and a 20X objective provides a magnification of 200X.
5. Practice moving the slide on the stage using the co-axial handle - forward and back, from left to right and vice versa.
6. Prepare a "wet mount" using a Daphnia from the collection of living materials and study it at 100X. Make a sketch of the organism on the blank sheet provided at the back of the manual for this purpose.

Identifying Animal Plankton - See next page

Study and sketch as many of the living specimens as time will permit. Identify these using reference books available and with the help of the instructors. Methocel (methyl cellulose) is available for you which will slow the organisms down so that they may be studied more easily. Make a tiny ring on the slide using the methocel and place a drop of the sample containing the specimens inside the ring.

Identification of Animal Plankton and Invertebrates

Protozoa - Single-celled organisms which may be seen as individuals or in colonial form. Have flagella, cilia or pseudopodia. See page 3-4 for types. Most forms lack the colour pigments which are present in algae.

Micro-invertebrates

Porifera - Fresh-water Sponges - are usually seen as a spongy growth on any stable substrate such as submerged twigs and intake screens. Microscopically they consist of great numbers of needle-like spicules which are interwoven to hold together a non-cellular matrix.

Coelenterata
- Hydra - an elongated cylinder with a basal attachment and a ring of tentacles at the distal (free) end.
- The tentacles surround the mouth and direct food to the interior hollow tube of the body.
- Daughter colonies called Buds may be present on the sides of the parent cell.
- Limited locomotion is achieved by summersaulting or gliding along the substrate.

Turbellaria
- Flatworms - elongated flat microscopic worm. Slightly larger than protozoa
- No body segmentation. Eye spots may be seen at one end which distinguishes the head
- Head may be pointed or triangular or flattened
- Oral cavity and anus are one and the same in mid-region of the body

Rotatoria
- Rotifers - Multicelled microscopic animals
- Highly organized internal structure including mouth parts, mastax, digestive tract and circulatory system
- two wheel-like crowns of cilia may be seen at the anterior end of the body
- Some species have a forked toe, others have a single posterior foot
- Some have a hard shell-like cuticle or lorica
- Others have a flexible telescopic trunk-like body
- The crown of cilia sort out the food to be eaten

- The mastax consists of two muscular jaws which operate like hammers which macerate the food.

Nematoda
- Roundworms - round slender worm-like form - but lack segmentation
- highly organized internal structure i.e. digestive tract, reproductive organs (not evident in flat worms)
- body diameter and length remains constant
- moves by continuous thrashing about of the body
- most individuals visible to the naked eye

Bryozoa
- Moss Animalcules - in the water they appear as a large gelatinous mass which may have a brown or green colour
- individuals coat the surface of the gelatinous mass and microscopically appear like cylindrical branched tubes with a crown of tenticles at the tips of the branches
- Statoblasts or resting cells are frequently seen on old colonies and are round or oval in shape with a border of anchor-like projections. Statoblast visible to naked eye

Annelida
- Oligochaeta - Commonly called aquatic earthworms or sludgeworms
- These are true segmented worms with setae on each segment. Digestive tract and mouth parts are evident on microscopic examination.
- Visible to the naked eye
- usually associated with polluted conditions

- Hirudinea - Leeches - are dorsalventrally flattened and visible without aid of microscope
- sucker type mouth
- muscular body is capable of contracting or expanding greatly
- eggs and cocoons may show up in water samples

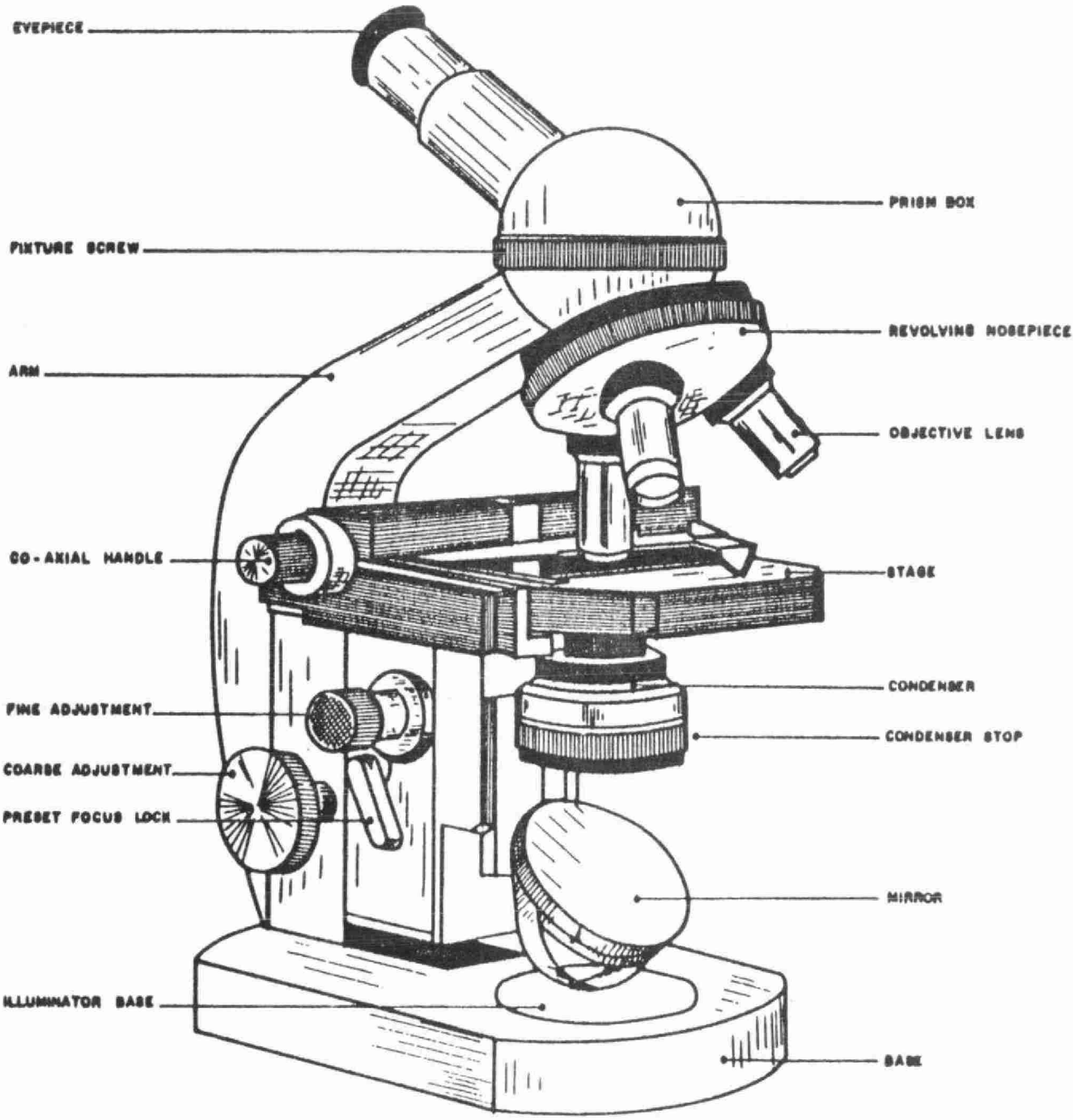
Micro-crustacea

Cladocera
- Daphnia or Water Fleas are usually visible to naked eye as little white specks
- single eye is apparent on microscopic examination
- one pair of branched antennae are used in locomotion (appears as a hopping or jumping motion)

	- Organism is enclosed in an outer skeleton or carapace - highly organized internal structure and appendages are present
Copepoda	- Cyclops - adults visible to naked eye - large branched antennae and highly branched swimming legs used for locomotion - single eye apparent Exoskeleton in form of a segmented and tapered shell - egg sacs may sometimes be seen attached to abdomen
Amphipoda	- Scuds or sideswimmers are large enough to be seen with naked eye - Body is laterally compressed and the two eyes are visible - There is a pair of appendages for each body segment, some of which are adapted for feeding, breathing, locomotion or reproduction - These organisms closely resemble their larger relative, the crayfish.
Insecta	- Tendipedidae - Midge larvae are commonly called Blood worms due to their bright red colour. They are not worms but the aquatic larval stages of true insects - They have segmented bodies with differentiated head and caudal segments.

NOTES

PARTS OF THE MICROSCOPE



CHARACTERISTICS OF MAJOR TYPES OF ALGAE

Algae, which are important in water supplies, may be placed in four general groups, the blue-green algae, the green algae, the diatoms and the pigmented flagellates.

1. BLUE-GREEN ALGAE

These are the simplest form of algae and are best described by what they do not have.

(i) They are the only algae in which the pigments are not localized in definite bodies but dissolved throughout the cell. Blue, red, or other pigments are present in addition to chlorophyll thus giving the cells a bluish green, yellow, or red colour, at least en masse.

(ii) No nuclear membrane is apparent.

(iii) No flagella (whip-like appendage to allow movement) is present.

(iv) Blue-greens tend to achieve nuisance concentrations more frequently in the warm summer months and in the richer waters.

(v) Vegetative reproduction, in addition to cell division, includes the formation of "hormogones", or short specifically delimited sections of trichomes (filaments).

(vi) Some species have specialized spores which carry the algae through periods when unfavourable environmental conditions are encountered. Spores of three types are encountered:

(a) "Akinetes" are usually long, thick-walled resting spores.

(b) "Heterocysts" appear like empty cell walls, but are actually filled with protoplasm and have occasionally been observed to germinate.

(c) "Endospores", also called "gonidia or conidia", are formed by repeated division of the protoplast within a green cell wall.

(vii) Many species of blue-greens have a definite gelatinous sheath or envelope surrounding each cell, colony or filament.

(viii) There are unicellular, colonial and filamentous species of blue-greens.

Examples: Anabaena (filamentous)

Anacystis (colonial)

Oscillatoria (filamentous)

Chroococcus (single celled or colonial)

2. NON-MOTILE GREEN ALGAE

The non-motile green algae constitute another heterogenous assembly of unrelated forms. In this classification system, the yellow-greens and desmids are included with the non-motile greens.

(i) They have a well-defined nucleus

(ii) They have well organized chloroplasts. These chloroplasts contain chlorophyll. The shape and position of the chloroplast is often distinctive. Pyrenoids are a distinctive characteristic of some members of this group.

(iii) They lack flagella or other locomotor appendages

(iv) They have a semi-rigid cell wall

(v) There is an extreme structural variation among members of this group

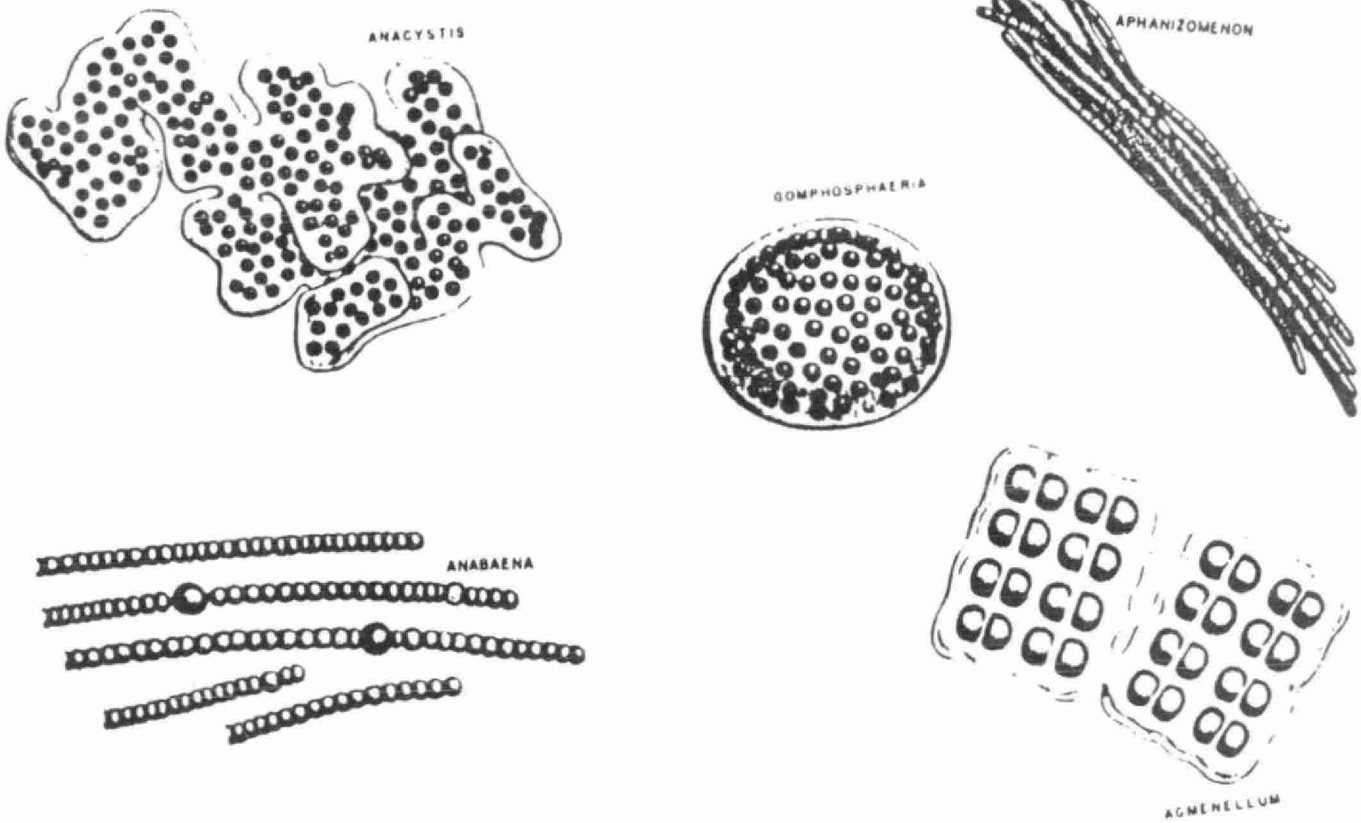
(vi) Some types tend to occur as a general planktonic mass or "bloom", often in combination with two or more species. Some examples are: Sphaerocystis (colonial), Pediastrum (colonial), Scenedesmus (colonial), and the desmid Closterium (single-celled)

(vii) Threadlike (filamentous) algae may form masses or blankets, cutting off light, and reducing water circulation. They also add considerably to the total mass of organic matter. Some examples of this type are: Spirogyra, Cladophora, Oedogonium, and Chara.

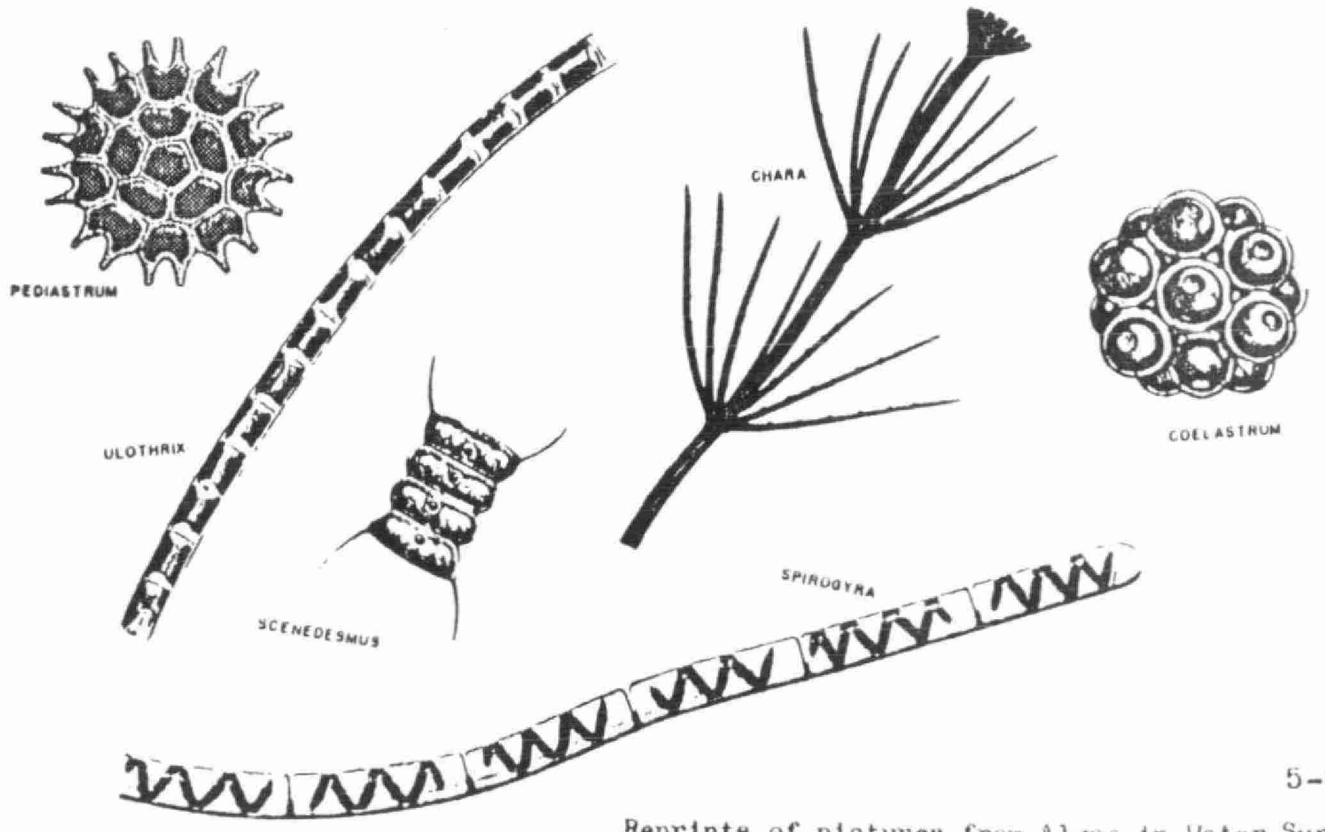
3. PIGMENTED FLAGELLATES

The "pigmented flagellates" (in contrast to the non-pigmented or animal-like flagellates) are a heterogeneous collection of motile forms from several different algal groups.

BLUE GREEN ALGAE

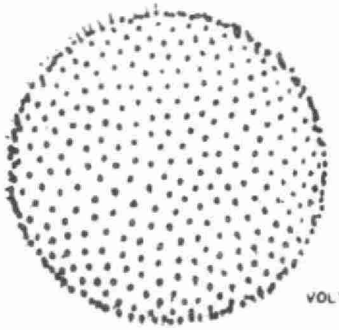


NON-MOTILE GREEN ALGAE (Including Filamentous)

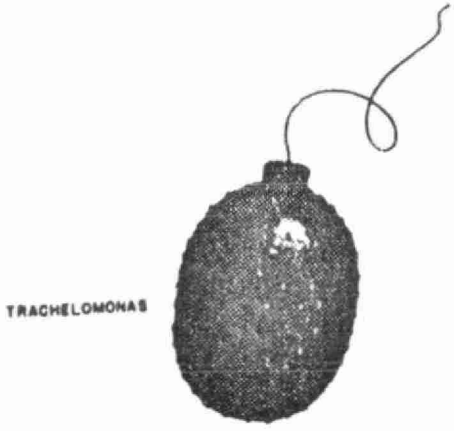


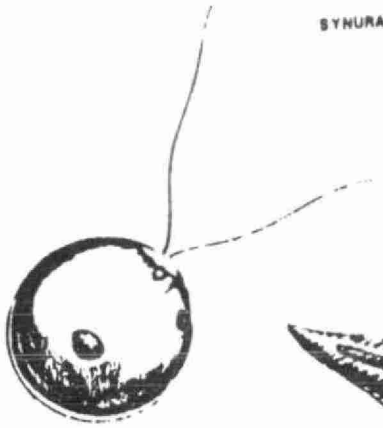
Reprints of pictures from Algae in Water Supplies
U.S. Department of Health, Education and Welfare

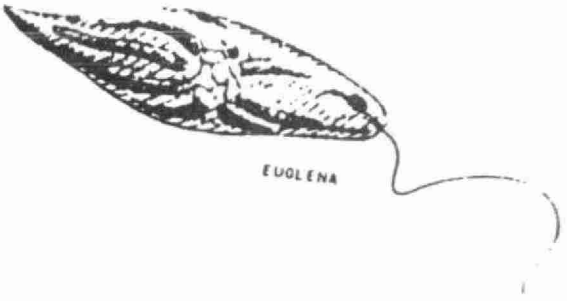
PIGMENTED FLAGELIATES


VOLVOX


SYNURA

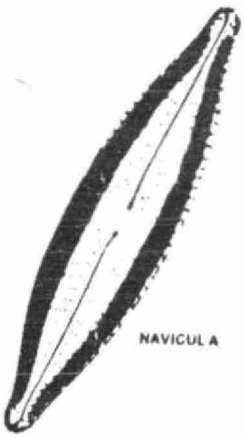

TRACHELOMONAS

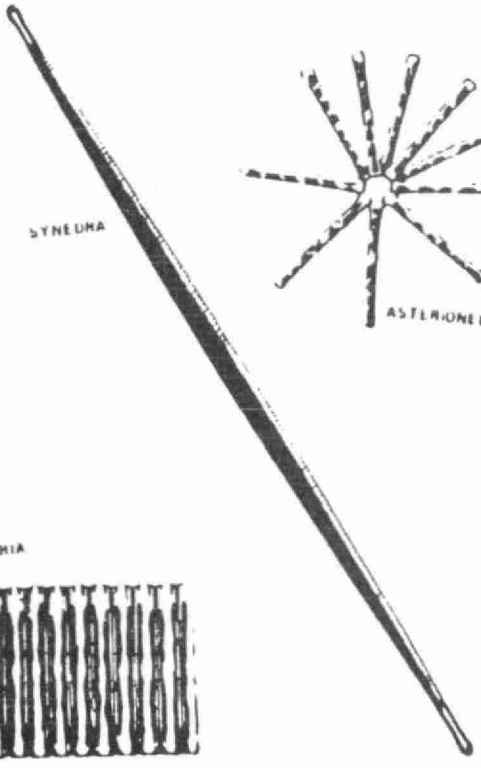

CHLAMYDOMONAS


EUGLENA

DIATOMS

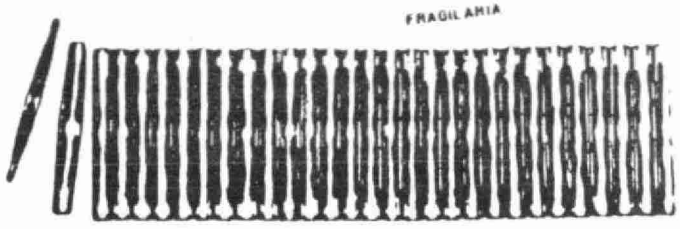

MELOSIRA


NAVICULA


SYNEDRA

ASTERIONELLA


TABELLARIA


FRAGILARIA

Reprints of pictures from Algae in Water Supplies
U.S. Department of Health, Education and Welfare

(i) They have one or more flagella per cell for movement

(ii) A thin membrane is usually present surrounding each cell

(iii) There is a well-defined nucleus.

(iv) A light sensitive eye-spot is usually present.

(v) The chlorophyll is contained in one or more distinctive chloroplasts. In addition to chlorophyll, other pigments may be present in the chloroplasts.

(vi) Non-motile life history stages may be encountered in some forms.

(vii) Masses of stored starch called pyrenoid bodies are often conspicuous in certain genera of this group.

(viii) Some examples are: Euglena (single-celled)

Synura (colonial)

Chlamydomonas (single-celled)

Dinobryon (single-celled or colonial)

Volvox (colonial)

4. DIATOMS

(i) In appearance, they are geometrically regular in shape. The presence of a brownish pigment in addition to chlorophyll gives them a golden to greenish colour.

(ii) Motile forms move slowly in a distinctive hesitating progression.

(iii) The most distinctive structural feature is the two-part shell (frustule) composed of silicon dioxide (glass).

(a) One part fits inside the other as two halves of a pill box, or a petri dish.

(b) The surface of these shells are sculptured with minute pits and lines arranged with geometric perfection.

(c) The view from the side is called the "girdle view), that from above or below, the "valve view".

(iv) A nucleus is present in all diatom cells.

(v) There are two general shapes of diatoms, circular (centric) and elongate (pennate). The elongate forms may be motile, the circular ones are not.

(vi) Diatoms may be single-celled, colonial or filamentous.

Examples: Navicula (single celled)

Melosira (filamentous)

Fragilaria (colonial)

Cyclotella (single celled)

Note: For a summary of the four main algal groups, see page 8 of Palmers Algae In Water Supplies.

BLUE-GREEN ALGAE

1. WHAT ARE THE BLUE-GREEN ALGAE?

The blue-green algae (Cyanophyta) comprise that large group of microscopic organisms living in aquatic or moist habitats, carrying on photosynthesis and having differentiation of cells. Thus blue-greens are a little more complex than bacteria and simpler than all other plants called algae.

2. CHARACTERISTICS

(i) The presence of the blue-green pigment, phycocyanin, produces the blue-green colour which is characteristic of members of this group. Chlorophyll is present in addition to phycocyanin and is distributed throughout the cell rather than being contained in chloroplasts.

(ii) Some blue-green forms are gelatinous masses of various shapes floating in water. Others, microscopic in size, grow in great numbers so as to colour the water in which they live. Structurally their cells are similar to bacteria. Their protoplasts may be sheathed or embedded in gelatin, making them slimy.

(iii) There are no organized nuclei in the cells of the blue-green algae, no vacuoles, flagella or other locomotor appendages.

(iv) These algae reproduce by asexual means such as fission (simple cell division) and the filamentous forms by fragmentation. In certain forms such as Oscillatoria, specialized separation discs may form in a portion of the filament and the section between these two discs, called a hormogone, will break away to form a new filament.

(v) Specialized cells are present in some species, such as akinetes, endospores and heterocysts. Akinetes and endospores may carry the algae through periods when unfavourable conditions are encountered. New filaments may sometimes be formed from heterocysts in some filamentous species such as Anabaena and Aphanizomenon.

3. SIGNIFICANCE OF BLUE-GREENS

They have both positive and negative economic significance. Because they can convert radiant energy into chemical energy, they are producers forming a first link at the base of the food chain. Because many very intricate nutritional relationships exist among the myriads of organisms, it is difficult to know the value of the blue-greens. When a "bloom" of blue-green algae develops, the algae sometimes

drift into bays or along beaches where it decomposes. As decomposition takes place, the mass of algae becomes unsightly, and creates foul odours and even toxins for some species. The decomposing algae are buoyed up to the surface of the water because "pseudovacuoles" or pockets of gas develop. There is usually a change to a yellowish colour as the algae degrade.

Some blue-green forms are able to clog pipes, filters and intake screens.

Some blue-greens live in association with other organisms as symbionts. Still others are found in polluted waters, because they are able to exist in habitats poor in oxygen. The growth of these kinds of algae under such conditions tends to make a polluted condition worse.

5. EXAMPLES OF BLUE-GREEN ALGAE

Most species of blue-greens may be placed into two major groups, the non-filamentous (coccoid) forms and the filamentous forms.

Anacystis

1. Colonies of Anacystis are always free floating.

2. There is a great variety in size and shape of the colonies of this genus(spherical or irregular, microscopic or macroscopic.)

3. The gelatinous matrix surrounding colonies of Ancystis may be extremely transparent and is easily broken up on preservation.

4. Individual cells of this genus frequently contain pseudovaculoles.

5. Anacystis includes Gloeocapsa and Chroococcus.

Anabaena

1. Filaments of Anabaena may occur singly, in irregular or in a free floating or delicate mucous matrix.

2. The trichomes have practically the same diameter throughout and often form straight, spiral or irregularly twisted loops.

3. The vegetative cells of Anabaena are spherical or bowl-shaped, rarely cylindrical and never discoid.

4. Heterocysts are present which are the same shape but are slightly larger than the vegetative cells. These cells are clear in colour in this genus.

5. The akinetes of Anabaena are larger than the vegetative cells and tend to be sausage-shaped.

6. This genus may produce an undesireable grassy, mouldy or septic odour.

7. Anabaena may be distinguished from Nostoc by the lack of a firm gelatinous envelope.

Aphanizomenon

1. Generally, cells of Aphanizomenon are smaller than in Anabaena, and trichomes are straight with cylindrical akinetes in evidence. The trichomes may be laterally joined into loose macroscopic free floating bundles which look like sheafs of wheat.

2. Cells are cylindrical or bowl-shaped and longer than they are broad.

3. Heterocysts of this genus generally do not occur at the end of the filament.

4. The akinetes are cylindrical and relatively long.

5. Aphanizomenon often imparts a grassy or nasturtium-like odour to the water.

Oscillatoria

1. Filaments may occur singly or are interwoven to form mats of indefinite extent.

2. Filaments are unbranched, cylindrical, and practically without sheaths.

3. Oscillatoria moves with a slow oscillating movement when living.

4. Species with narrow filaments have long cylindrical cells while those with broader filaments have short broad cells.

5. Oscillatoria has no akinetes or heterocysts. Hormogonia are formed and break away to reproduce by fragmentation.

6. Species of Oscillatoria may be readily distinguished from Lyngbya by the absence of a sheath.

Nodularia

1. Vegetative cells, heterocysts, and even the akinetes are broader than long.

2. Trichomes are practically the same diameter throughout.

3. Sheaths are usually distinct, fairly firm, and with a single trichome.

Lyngbya

1. This is similar to Oscillatoria but has a firm relatively thin, hyaline to yellowish-brown, homogeneous or lamellated sheath which encloses but a single trichome and generally projects for some distance beyond it.

2. The filaments are cylindrical and either straight or twisted into regular spirals.

3. The filaments may be grey, pale to bright blue-green, or variously coloured.

Agmenellum

1. The cells are arranged in flat plates in a gelatinous envelope. The cells are regularly arranged in vertical and transverse rows.

2. Small colonies are usually perfectly flat; large colonies, although one cell in thickness, are usually more or less bent and distorted.

Gomphosphaeria

1. Colonial form, oval or globular in appearance.

2. Cells usually arranged in a single layer about the periphery of the clear mucilaginous envelope and joined to strands which radiate out from the centre of the sphere. Some cells may appear closer to centre of colony as strands are not all equal in length.

3. Individual cells may be spherical or heart-shaped. Heart shaped formation is caused by fission process by which cells divide.

4. Cells contain a homogenous chloroplast which gives it a bright blue-green colour which may change to brown or grey with the increase in the presence of pseudovacuoles. Should be compared to Coelosphaerium.

LABORATORY: THE IDENTIFICATION OF BLUE-GREEN ALGAE

1. Using the key in "Algae in Water Supplies" endeavour to determine the genus to which the sample of algae provided by the instructor belongs. Even if you know the algae, follow through the key for practice.

2. Examine the preserved materials to become familiar with the genera available and terminology related to classification. Make sketches for future reference.

3. Examine the prepared slides Aphanizomenon, Anabaena and Anacystis under both low and medium power. Note the heterocysts and akinetes in Aphanizomenon and Anabaena.

NOTES

NON-MOTILE GREEN ALGAE - FILAMENTOUS AND COCCOID

FILAMENTOUS GREEN ALGAE

Filamentous green algae may be several inches or even a foot or more in length. In many cases they are not found as isolated filaments but develop in large aggregations to form floating or attached mats or tufts. The attached forms are generally capable of remaining alive after being broken away from the substrate. Included in this group are some of the most common and most conspicuous algae in fresh-water habitats. A few of them have been given common names such as pond silk, green felt, frog-spawn algae, and stoneworts.

1. CHARACTERISTICS

1. These algae are composed of cells held together end to end in filaments which may either be attached or free floating. In some green algae, the filaments are branched and in others they are not. Gelatinous envelopes are present in some species. Filaments may or may not taper towards the tips.

2. Each cell is either a short or long cylinder with a distinct wall, and contains a nucleus which is seldom readily apparent.

3. A chloroplast (plastid) is the most outstanding structure present and contains the pigment chlorophyll. The chloroplasts which are essential for food production vary in size, number, and shape in different algal forms. They may be "parietal" i.e. they lie close to the outer cell wall; others are "axial" i.e. they extend through the central axis of the cell. Some chloroplasts, as in Spirogyra and Zygnema are very distinctive and render identification possible on this feature alone.

4. Clean areas of cell sap ("vacuoles") are generally present in the green algal cells.

5. Attached green filaments have the basal cell developed into a "hold-fast cell" (hapteron).

6. Reproduction in filamentous green forms may take place by several methods:
   (a) Fragmentation of filaments may occur.
   (b) Many kinds reproduce sexually, often with specialized gamete-forming cells.
   (c) Zoospores (motile) and aplanospores (non-motile) are common.
   (d) Cell division may occur in all cells or in certain selected ones.

2. SIGNIFICANCE OF THE FILAMENTOUS GREEN ALGAE

1. Filamentous greens may promote development of animal plankton in storage reservoirs and lakes by providing the proper growth conditions for micro-crustaceans such as Daphnia, Cyclops, etc.

2. Green algal forms may clog filters and intake screens at water treatment plants.

3. Foul odours may develop where these algae are washed ashore (e.g. Cladophora problem - Lake Erie and Lake Ontario). Large masses of Cladophora may wash ashore where it decomposes; thus interfering with the recreational values of the land.

4. Filamentous greens have been known to foul fishing nets. (i.e. Cladophora - Bay of Quinte).

5. Green algae may produce a slime which intereferes with some industrial uses of water such as in paper manufacturing and in cooling towers.

6. Green algal forms, along with other algae help to purify streams and maintain a favourable oxygen balance.

7. Some green algae are useful as indicators of water quality in relation to pollution.

3. EXAMPLES OF FILAMENTOUS GREEN ALGAE-UNBRANCHED FORMS

Spirogyra

This plant is characterized by the presence of spiral chloroplasts which make up a large proportion of the contents of the cell. Pyrenoids, which are centres of starch formation, are arranged along the chloroplasts.

Ulothrix

1. This algae has a single parietal chloroplast in each cell. The curled edges of the chloroplast are usually evident. Sometimes confused with diatom Melosira

2. Some species have cylindrical cells and others have cells shorter than they are wide.

Mougeotia

1. The presence of a plate-like axial chloroplast signifies members of this genus. The chloroplast sometimes shifts its position so that it appears as a narrow ribbon.

2. Chloroplasts of narrow cells have two, three, or more pyrenoids arranged in a linear series; chloroplasts of broad cells have several irregularly arranged pyrenoids.

3. Depending upon the intensity of illumination, the chloroplasts lie at right angles to, or parallel with, the incident rays of sunlight.

4. Chloroplasts of several successive cells usually have the same orientation, but sometimes the chloroplast of a single cell is so twisted that opposite ends are at right angles to each other.

5. The cell contains a single nucleus midway between the poles, and it lies flattened against the chloroplast.

Zygnema

1. A pair of star-shaped chloroplasts signifies members of this genus. A large central pyrenoid is always present in each of the chloroplasts. Application of an iodine solution facilitates observation of the chloroplast.

Oedogonium

1. Sterile specimens of Oedogonium may be recognized by the unbranched filaments of cylindrical cells. Certain cells have tranversely striate walls at the distal end.

2. The basal cell of a filament is modified to form a holdfast, and the apical cell is usually broadly rounded.

3. Cells of Oedogonium are sometimes slightly enlarged at their ends, and have straight sides.

4. Cell division is either terminal or else intercalary and it may take place in any cell but the basal one.

5. The cells are uninucleate and have a single reticulate chloroplast completely encircling the protoplast.

6. The chloroplast usually has many pyrenoids, one at each of the larger intersections in the reticulum.

4. BRANCHED FORMS

Cladophora

1. This genus is characterized by a spreading branching effect with its cells generally cylindrical in shape. It is a relatively large microscopic form.

2. The ends of filaments taper very abruptly, with only the terminal cell being involved.

Stigeoclonium

1. This genus resembles Cladophora somewhat, but the tapering off of filaments is more gradual, involving two or more cells.

Chaetophora

This genus resembles Stigeoclonium but is surrounded by a gelatinous matrix.

SPECIALIZED FORMS

Chara

These are large macroscopic plants which grow erect with stem-like branches which are arranged in whorls and bear forked leaves. Chara usually feels rough because lime is encrusted on it.

Hydrodictyon

Cells of this alga form a network and is commonly called "water-net" on this account.

NON-MOTILE GREEN ALGAE - COCCOID

The coccoid algae are those that exist as free-floating planktonic units. Some non-motile coccoid forms tend to grow in masses or mats of material, either attached or free-floating.

1. CHARACTERISTICS

1. Since green forms contain a good deal of starch, a deep purple colour will be produced when treated with iodine solution.

2. Cells of some green species often link together to form coenobes. A coenobe is a colony of cells which does not multiply during the life of the colony although the cells do increase in size.

3. There is a great variety in size and shape among the members of this group. Cells or colonies may be round, irregular and often are ornate.

2. SIGNIFICANCE OF COCCOID GREEN ALGAE

1. Coccoid green algae may cause raw water supplies to be odourous and may contribute to filter clogging.

2. These algal forms may assist in maintaining a favourable dissolved oxygen balance in the water.

3. These algal forms are important items in aquatic food chains.

3. EXAMPLES OF COCCOID GREEN ALGAE

Chlorella

1. Cells are small and generally spherical in shape.

2. A single parietal chloroplast is present.

3. Chlorella pyrenoidosa is often found in organically enriched waters. A dominance of Chlorella species is considered in some places to be an indication that a sewage stabalization pond is functioning to maximum capacity.

Scenedesmus

1. This genus is composed of a number of cells arranged with their long axis parallel to form a flat plate.

2. Scenedesmus forms a coenobial colony of up to 32, but usually 4 to 8 cells.

3. The appearance of cells of this genus may vary considerably with the species.

Pediastrum

1. Colonies (coenobial) of Pediastrum are free floating with up to 128 polygonal cells arranged in a single plane. There may or may not be spaces between the cells. The colonial shape of this genus usually resembles a gear-wheel.

2. The peripheral cells of each colony may differ in shape from the interior cells.

3. The exact arrangement of the cells of this genus seems to depend largely on the chance distribution of the original motile swarming zoospores at the time the coenobe was formed.

Cosmarium (desmid)

1. Cells of this genus are almost as wide as they are long and have a deep constriction across the center of the cell called the "isthmus" which forms two semi-cells.*

2. Cosmarium botrytis is reported in plankton from water supply reservoirs.

3. Some species have been reported to be sufficiently resistant to chlorine to penetrate rapid sand filters and may occur in distribution systems in considerable numbers.

3. EXAMPLES OF COCCOID GREEN ALGAE

Chlorella

1. Cells are small and generally spherical in shape.

2. A single parietal chloroplast is present.

3. Chlorella pyrenoidosa is often found in organically enriched waters. A dominance of Chlorella species is considered in some places to be an indication that a sewage stabalization pond is functioning to maximum capacity.

Scenedesmus

1. This genus is composed of a number of cells arranged with their long axis parallel to form a flat plate.

2. Scenedesmus forms a coenobial colony of up to 32, but usually 4 to 8 cells.

3. The appearance of cells of this genus may vary considerably with the species.

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*NOTE - A distinctive group of green algae characterized by a median constriction dividing the cell into two bi-laterally similar halves is known generally as a "desmid". (Closterium and Penium do not have this constriction). Each half of the cell is known as a "semi-cell". The nucleus lies in the "isthmus". Extremes of ornamentation and structural variety exist. Most are unicellular, but a few are filamentous or have the cells associated in shapeless colonies. They are found sparingly in the plankton almost everywhere but predominate in acid waters.

Closterium -(desmid)

1. Cells of this genus are small in size; no median constriction is present. The cells taper slightly but are not sharply pointed.

2. Closterium cells have a slight crescent shape curvature in their long axis.

Schroederia

1. Members of this genus are solitary, and free floating. Individual cells are long and pointed at both ends and often form the shape of an "S".

2. In some species, spines may be present which protrude from the ends of the cells.

Selenastrum

1. Cells of this genus are pointed at both ends, and bent so that their tips approach each other.

2. Cells may occur in groups of 4-16. Also groups of cells may associate to form masses of a hundred or more cells.

Kirchneriella

1. Cells of this genus resemble Selenastrum but are much broader and bent into a definite "c".

2. Cells usually occur in groups of four to eight in a broad, homogenous, gelatinous matrix.

Actinastrum.

1. Actinastrum colonies are composed of 4, 8, or 16 elongate cells which radiate in all directions from a common center.

Sphaerocystis

1. Colonies of Sphaerocystis are free floating and almost always with a perfectly spherical, homogeneous, gelatinous envelope.

2. Up to 32 cells may be included in a single colony

3. Sphaerocystis scheoeteri is the only species recorded and is present in the plankton of lakes and reservoirs.

Coelastrum

1. Cells of this genus form coenobial colonies of up to 128 cells.

2. Both the cells and the colony are generally spherical in shape. Individual cells in the colony are connected by protoplasmic processes of varying length.

3. This genus may be differentiated from Sphaerocystis by not having a surrounding gelatinous envelope.

Micractinium

1. Cells of this genus are spherical to ellipsoidal and may be united in irregular 4-celled coenobial colonies.

2. The free surface of each cell in a colony bears from one to seven slender hairs or setae.

Crucigenia

1. Cells of this genus forms free floating four-celled coenobial colonies. The colonies may be solitary or joined to one another to form plate-like coenobes of 16 or more cells.

Oocystis

1. The cells of Oocystis may be solitary, or up to 16 cells. Each cell or group of cells is surrounded by a partially gelatinized, expanded cell wall.

2. Individual cells are ellipsoidal to almost cylindrical.

Dimorphococcus

1. Cells of Dimorphococcus are arranged in groups of four. These tetrads are united to each other in irregularly shaped free floating colonies by the branching remains of the old mother-cell walls.

2. Two shapes of cells are normally found in each tetrad (the name suggests this). Two longer ovate cells end to end and a pair of shorter C-shaped cells on either side are present in each tetrad.

Staurastrum (desmid)

1. These desmids are radially symmetrical. Nearly all species have a deeply constricted isthmus.

2. Individual cells of this genus may be smooth, ornamented, or spined in a variety of ways.

3. Long truncated processes extend from the cell body.

Ankistrodesmus

1. Cells are long, slender and taper to a long point at each end.

2. Cells of this genus may be straight, curved, or twisted into loose aggregations.

3. Ankistrodesmus falcatus is often found in the plankton in water supplies and is considered to be one of the forms indicative of clean waters.

LABORATORY - NON-MOTILE GREEN ALGAE

1. Using the key in "Algae in Water Supplies", attempt to identify the alga provided by the instructor.

2. Draw a typical cell of Spirogyra using 450X, labelling all of the parts which make up the protoplasmic content of the cell.

3. Examine the slide preparations of green algae which are available, making sketches showing details of their structure for future reference. Sketch at least two coenobial types of green algae.

NOTES

FLAGELLATED ALGAE

1. CHARACTERISTICS

1. All flagellates possess one or more flagella per cell. A flagellum is a whip-like appendage which acts as a propeller.

2. Some flagellates are unicellular, others are colonies of cells held together in gelatinous envelopes.

3. One or more chloroplasts are present in each cell. These chloroplasts contain chlorophyll.

4. Thick-walled resting stages may be assumed by certain species if unfavourable conditions are encountered.

2. SIGNIFICANCE OF FLAGELLATES

1. Many pigmented flagellates can produce strong tastes and odours when they are present in water supplies. The flagellated alga Synura in small numbers can impart a perceptible cucumber odour to the raw water. Bacteriological doses of chlorine may aggravate the situation by changing the nature of the odour from cucumber to fishy or oily.

2. Certain pigmented flagellates are able to withstand polluted conditions. These forms tend to indicate polluted conditions if other types which will not tolerate pollution are not present.

3. Large numbers of certain flagellates may cause filter-clogging problems.

4. Flagellates release oxygen to the water and utilize carbon dioxide.

3. EXAMPLES OF FLAGELLATED ALGAE

Euglena

1. Cells of this genus are elongated, cylindrical or spindle-shaped and bear a single flagellum. Round forms of Euglena may occasionally be observed.

2. This form stores its food as paramylum (an insoluble carbohydrate) in numerous rod-shaped bodies.

3. A number of disc-shaped chloroplasts are present in the protoplasm of this genus.

4. Members of this genus are characterized by a red eyespot which is sensitive to variations in light intensity.

5. Because of the presence of the pigment haematochrome, some species of Euglena appear red in colour. With "bloom" conditions of Euglena sanguinea the entire surface of a pond will appear to be covered by a bright red film.

Chlamydomonas

1. Chlamydomonas is a solitary free-swimming genus. Cells with two flagella are usually round or oval in shape.

2. A single cup-shaped chloroplast is present.

3. Some species are characterized by a gelatinous sheath.

4. Members of Chlamydomonas are often found in oxidation ponds and polluted waters.

Carteria

1. This genus closely resembles Chlamydomonas but has four flagella instead of two.

Phacus

1. Cells of this genus are often flattened and twisted, with a pointed tip or tail end.

2. The cell wall is marked with fine ridges.

3. This genus is characterized by one or more do-nut rings of paramylum.

4. Some forms such as Phacus pyrum are favoured by polluted water.

Trachelomonas

1. The protoplasm of this genus is enclosed in a brown shell called a "lorica "or "test" which may be oval or flask-shaped. This test has a hole or collar through which a single flagellum protrudes.

2. The surface of the test is usually brown in colour, and may appear smooth or rough.

3. Some species of this group have been known to clog filters.

Gonium

1. Gonium colonies typically have 4 to 32 cells arranged in a plate. The individual cells are embedded in a gelatinous matrix.

2. Sixteen-celled colonies move through the water in a sommersault fashion while four and eight celled colonies swim flagella-end first.

Pandorina

1. Colonies of Pandorina range up to 32 cells. The colony is generally spherical.

2. The individual cells of this genus are arranged in a hollow sphere within a gelatinous matrix.

Eudorina

1. This spherical colony has up to 64 cells. The individual cells are deeply embedded in a gelatinous matrix. This genus is common in soft water lakes while Pandorina is more often encountered in hard water lakes.

Volvox

1. This colonial form rarely has less than 500 cells per colony.

2. The central portion of the mature colony may contain only water. Daughter colonies of Volvox form inside the parent colony.

Ceratium

1. Ceratium is a member of the dinoflagellate group (armoured flagellate). This genus is distinctive in that one anterior and two posterior ends are continued as long horns. Members of this genus have a very distinctive form.

2. This genus is brown in colour.

3. Each cell of this group has a transverse groove and two flagella. One flagellum lies in the transverse groove.

4. Seasonal changes in temperature have a pronounced effect on the shape and number of these algal forms.

5. Ceratium hirudinella in high numbers has been reported to impart "vile stenches" to the water.

Peridinium

1. The cell walls of this group are thick, heavy, have a transverse groove and are usually highly ornamented.

2. Some members of this group may impart a fishy odour to the water.

Mallomonas

1. This is a solitary, free-swimming genus with one flagellum.

2. Each individual cell is covered with silicious plates, many of which bear long spines.

Synura

1. This is a colonial form.

2. Each pyriform-shaped cell has two flagella. The individual cells are radially arranged in the colony. Each cell may be characterized by the presence of two elongated, slightly bent chloroplasts.

3. Synura in very low numbers may impart a definite cucumber-like odour to the water.

Dinobryon

1. Cells of Dinobryon may be solitary, colonial, free-floating or attached.

2. Each cell is attached to the bottom of a lorica that has a closed, pointed base and an open, cylindrical or somewhat flaring apex.

3. Each cell of this genus has two flagella of unequal length.

4. This genus causes a fishy odour in the raw water.

LABORATORY - PIGMENTED FLAGELLATES

1. Examine some of the living flagellates in the samples provided, noting their corkscrew-like movement. Note how Euglena is able to change shape.

2. Follow through the key to identify Euglena. Make a sketch of this organism under medium power, slowing down the organisms with methocel so that the principal parts may be noted and sketched.

3. Examine the various prepared slides of the flagellates which are available and make sketches to demonstrate the features by which they may be identified.

NOTES

PLANKTON SAMPLING PROGRAMS

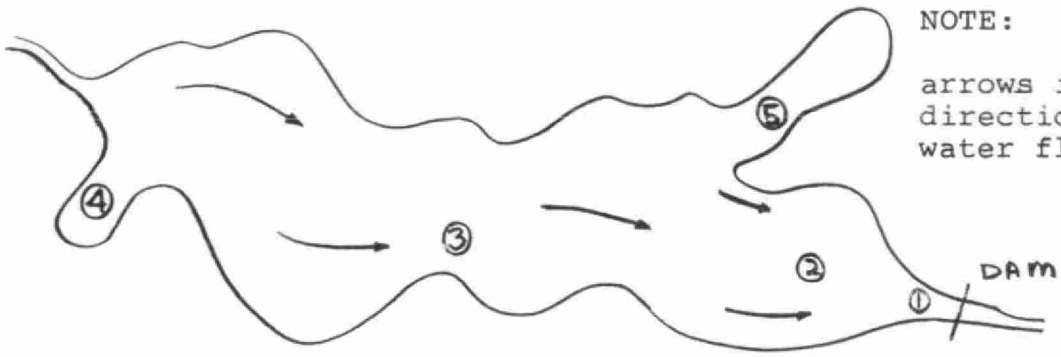
1. An organized program of plankton counting should be based on samples taken at least once a week.

2. Seasonal patterns of algae development tend to repeat themselves year after year and so are relatively predictable - a certain amount of seasonal variation is to be expected, however (variables such as nutrients, rainfall, temperature).

3. More analyses may be desirable if population of a troublesome species increases.

4. It may be easier and less costly to control an upsurge of one particular alga in its early stages.

Location of Sampling Points



1. Both shallow and deep samples should be taken.

2. Samples from top to bottom at one sampling point may be composited to provide a summary for that station.

3. Each major bay or shoal area of a reservoir should be sampled and one should be taken near an intake if the latter is present.

4. Sampling over 24-hour periods or longer may be helpful either studied individually or composited.

Preserving Samples

1. Samples submitted to the laboratory must be preserved.

2. Formalin at 3 - 5% causes shrinkage of cells and can cause flagellates to explode if these are in the sample - use no more than 3% formalin for any sample.

3. Lugols solution is sometimes used. It is a mixture of potassium iodide (10 gms) and iodide crystals (5 gms) dissolved in 100 ml distilled water.
   The amount required to preserve a sample is that amount which will give the sample a deep amber (tea) colour.

4. A minimum of preservative may be used to render organisms immobile without altering the physiology of the cell too much.

5. Because some flagellated forms are delicate and destroyed when a preservative is added, it may be desirable to obtain a duplicate sample, one preserved and the other unpreserved from the same sampling location.

6. A .1% merthiolate solution will adequately preserve plankton up to six weeks.

DIATOMS

1. CHARACTERISTICS

1. Diatoms have rigid cell walls which are made of silicon dioxide (silica or glass). The cells contain chromatophores and have a brown pigment in addition to chlorophyll. The individual cells or cell walls of this group are called frustules.

2. Diatoms may be unicellular, filamentous or colonial.

3. Each cell resembles a pillbox - two separate parts with one overlapping the other. The "valve" view is that of the top or bottom of the box. The "girdle" view is the side view.

4. Cells are either "pennate" which are elongated in structure or "centric" which affords one circular view.

5. Diatoms reproduce mainly by cell division although formation of gametes do occur.

6. Cell markings are evident such as the "raphe" or pseudoraphe which extends longitudinally, and "striae" or "punctae" which are lines of pores extending from the raphe or pseudoraphe to the margin. "Nodules" at the ends of the raphe may also be present and internal shelves called "septa" are another feature.

7. Some diatoms can move slowly by a process called protoplasmic streaming.

2. SIGNIFICANCE OF DIATOMS

1. Diatoms are the most important group of algae which cause filter-clogging. The most serious offenders are Asterionella, Fragilaria, Tabellaria and Synedra. The rigid silica wall of diatoms is not subject to decomposition. Therefore, even though the diatoms may die off rapidly on the surface of the filter, their silica walls remain to plug the pores in the sand.

2. Some diatoms such as Asterionella can produce tastes and odours in raw water supplies.

3. Water quality can be evaluated by specialists who understand the effects of polluted conditions on numbers of different diatoms.

3. EXAMPLES OF DIATOMS

Pennate Diatoms

Synedra

1. The frustules of this form are much longer than they are broad. (symmetrical)

2. Synedra may occur as a solitary cell (most often) or in radially-arranged colonies.

3. The ends of each frustule are sometimes pointed, swollen or blunt.

4. A pseudoraphe is present.

5. Because Synedra is motile, it is capable of penetrating deeply into sand filter beds.

Fragilaria

1. These cells appear linear to fusiform in the valve view but are rectangular in the girdle view. (symmetrical)

2. Cells of this genus unite to form a colony. The cells appear as a number of "cigars" stacked on top of each other valve to valve.

3. A pseudoraphe is present.

Tabellaria

1. Cells of this genus unite in zigzag chains.

2. Each cell is long and slightly inflated at the centre and at each end.

3. A narrow pseudoraphe is present.

4. Longitudinal septa are present inside each cell of this group.

Navicula

1. Cells of this genus are symmetrical, elongate, and often boat-shaped. The cells appear rectangular in the girdle view.

2. Each cell has a raphe with central and polar nodules.

3. Members of this group are generally single-celled and free floating.

Nitzschia

1. Cells of this group are generally elongate as seen in the valve view. The sides of each cell may be constricted at the centre.

2. A true raphe is present.

3. On each valve, transverse striae or punctae are apparent.

4. Members of the genus Nitzschia are generally single-celled.

Asterionella

1. Individual cells are joined together in star-shaped colonies.

2. The inflated ends at the centre of the star are broader than at the free ends.

3. Cells of this genus should be compared to Tabellaria and Diatoma.

Surirella

1. Cells of this genus are elliptical or oval-shaped with rounded ends. (asymmetrical).

2. Surirella occurs as relatively large elongated elliptical cells with heavy transverse costae.

Meridion

1. Individual cells of this genus are wedge-shaped in girdle view and form fan-shaped colonies which are joined valve to valve.

2. Members of this group have internal septa which show through the wall of the frustule.

Cocconeis

1. Frustules of this diatom are broadly elliptical in the valve view and transversely curved in the girdle view. The two valves are similar in outline but dissimilar in structure.

Pinnularia

1. The symmetrical frustules of Pinnularia have valves that are usually with rounded poles and straight parallel sides.

2. Frustules of Pinnularia are usually solitary and free floating.

Gomphonema

1. This genus is to be distinguished from other naviculoid diatoms by having frustules that are transversely asymmetrical in both valve and girdle views.

2. The valves are straight with one pole broader than the other.

3. Frustules of Gomphonema are usually epiphytic and present on filamentous branching algal forms. Sometimes the frustules are sessile.

CENTRIC DIATOMS

Cyclotella

1. Cells of this genus are circular in the valve view. A smooth region in the centre and a peripherial lined region characterizes the valve view of this form.

2. Cyclotella is generally solitary and free floating.

Stephanodiscus

1. Cells are circular in the valve view.

2. The rows of puncta on the surface of the valve extend into the centre of the cell.

3. Some species of this genus are characterized by the presence of spines which extend from the wall of the frustule.

Melosira

1. The cells of Melosira are cylindrical, sometimes with convex valves.

2. Some species such as Melosira granulata have terminal cells which have robust spines. These spines or teeth assist in holding the individual cells together in a filament. These filaments can be quite long.

3. Melosira at low magnification appears similar to the filamentous green alga Ulothrix.

LABORATORY - DIATOMS

1. Using some of the micro-strainer waste which is available, make up a slide and observe under low power.

2. Note the varied forms which are present, their colour etc., and by pressing lightly on the coverslip, attempt to change the position of individual forms so that different views of the cells may be presented.

3. Examine the prepared diatom mounts and identify as many genera as you are able. The markings on the valve and girdle views should be apparent.

4. Note the presence of a true raphe in Navicula and a pseudoraphe (false raphe) in Synedra. The pseudoraphe is formed by interruptions in the lines of dots (punctae) which run transversely across the cell.

5. Make drawings of Stephanodiscus and Navicula under medium power, identifying and naming as many of the structural features as possible.

6. Study and make sketches of as many diatoms as possible, using the reference material available to identify the different genera, with the help of the instructors present.

NOTES

PREPARATION AND ENUMERATION OF PLANKTON

Preparation

1. Whenever possible samples should be studied initially without a preservative being added. Certain of the flagellate forms will either disappear entirely or be severely distorted by formalin as low as 1%. Preservative may be added to kill motile forms for counting after their identity has been established.

2. Unpreserved samples may be refrigerated for future analysis but if they are to be held more than a couple of days, preservative should be added. Commercial formalin is used to make a 3% solution or a more gentle preservative is merthiolate which need only be added to make a 1% solution.

3. Identification and enumeration equipment which is essential includes:

   (1) Compound microscope having: (a) mechanical stage
   (b) 10X ocular fitted with a Whipple ocular micrometer or reticule which is used to delineate the area to be counted and to measure the areal size of individual cells or pieces of algae.
   (c) Objectives: approx. 10X
   " 20X
   " 40X
   " 100X

   (2) Sedgwick-Rafter counting cells are essential which measure 50mm x 20 mm x 1mm, so that they will hold exactly 1 ml. of sample. Special cover glasses for this counting cell are provided.

   (3) The S-R cell is filled by drawing slightly in excess of 1 ml. of the sample into a pipette after the sample has been well shaken and allowing the sample to flow into one of the openings created by laying a cover glass diagonally across the counting cell. If this is done properly the air will escape from the opening on the opposite side and the cover glass will rotate into proper position by itself to cover the cell. Excess water can be removed by wiping lightly with a soft tissue.

   (4) After filling the S-R cell, it should be allowed to sit on the microscope stage for several minutes to allow the organisms to settle out.

   (5) If many blue-greens are present, iodine may be added to encourage these algae to settle since they tend to float at the surface.

Enumeration

1. Qualitative and Quantitative Analyses

It may only be necessary at times to determine what types of plankton are present and to obtain an approximate idea of their relative numbers. Perhaps a taste and odour problem has developed and it is only essential to determine what particular alga is causing the problem.

However, for analyses to be of lasting value, they must provide a relatively accurate measurement of the numbers of plankton for each genus present. If annual records are maintained, predictions may be ventured since similar conditions tend to develop in cyclic fashion year after year. However, seasonal variations do occur because of factors such as water temperature and amount of sunshine. Information related to water temperature, nature of the weather and cloud cover, water turbidity and pH is all valuable and may lead to more knowledgeable interpretations of what has happened in the past and what may be expected in future. It is only after records have been maintained for several years that accurate forecasting may be a certain accomplishment.

Regular quantitative counts should be made weekly at least. This should provide adequate forewarning of the increase of one particular alga to nuisance proportions so that remedial measures may be implemented before the nuisance condition is an established fact.

The Nature of the Quantitative Count - The Areal Standard Unit Method

The "clump count" has been used in the past but the major disadvantage of this simpler method is that filaments and colonies are counted as units, equal to individual cells. No cognizance is taken of the relative masses of the various organisms which are present.

Procedures related to the areal standard unit method which we will use are as follows:

(1) A Sedgwick-Rafter cell is filled with 1 ml. of the sample. The sample may or may not have to be concentrated depending on the density of organisms in the sample. Concentration is usually necessary. Procedures for concentrating are outlined in the next section.

(2) The areal values of algae in one or two strips across the cell are recorded, or perhaps the areal values for organisms present in a predetermined number of fields, (for a concentrated sample) and using appropriate multiplier factors, these areal values are projected to areal standard units per ml., litre, etc. An areal standard unit is 400 square microns.

(3) The area which needs to be examined varies with the concentration of algae in the sample. Generally, ten fields are enumerated in a concentrated sample. If the sample is not concentrated because of high numbers of algae, one or two strips are covered.

(4) Half of the cell should be checked using low power to determine whether rotifers or other animal plankton are present in the sample. In this "survey count" the actual numbers of organisms are recorded instead of their areal values.

(5) Before areal standard unit counts can be attempted, the microscope must be calibrated so that the linear and areal values of the lines and squares associated with the ocular micrometer are known (outlined in Section 16 - Calibration).

(6) Before starting the count the sample should be scanned to determine what organisms are present and to establish average areal values for types of algae which are abundant and relatively consistent in size. It may be necessary to use the medium power objective to determine the areal value of individual organisms more accurately. If this is the case, an ordinary slide must be used and it might be necessary to use the Sedgwick-Rafter concentrating funnel to obtain a sufficiently concentrated sample for this purpose. (See page 15.6)

(7) Records should be kept of the average areal values of individual species to avoid needless repetition of preliminary measuring. Different species of the same genera may be recorded as Species A, B, C, etc., with their distinguishing features being noted.

(8) For the first while it will be necessary to measure each cell, filament or colony which is encountered to determine its areal value. The individual areal values assigned for each genus are recorded on the bench sheet(p.18.3). When the count is completed, the total areal value for each genus is obtained, and is multiplied by the appropriate factor to obtain the number of areal standard units/ml.

CONCENTRATION TECHNIQUE FOR ALGAE ENUMERATION

Due to several factors such as too few organisms per field, occasional large organisms in isolated fields, clumping, etc., unsatisfactory algae counts can be obtained. To increase the accuracy of algae counts, it is essential to employ a concentration technique by which a large sample volume is reduced to a few milliliters. It is then possible to enumerate organisms in a relatively small number of fields rather than covering an entire strip or perhaps half the cell volume. An appropiate concentration factor must be inserted in the formula to calculate the quantity of algae present per ml. of sample.

Methods and materials

1. A 500 ml. Sedgwick-Rafter funnel is required for the concentration. The bottom of the funnel has a one-holed rubber stopper fitted with a glass U-tube which is connected by a rubber tubing to a suction apparatus. A 200 mesh silk bolting cloth disc 16 mm. in diameter is held tightly in place above the stopper and white Banding Sand is added to bring the level of the sand up to the "0" graduation mark of the funnel. The sand acts as a filtering medium.

2. Wet the sand with 5-10 mls. of distilled water to drive out the air.

3. Gently agitate the sample to be concentrated. Measure out 250-1,000 mls. (depending upon the visual density of the organisms in the sample) into a graduate cylinder.

4. Slowly pour the sample into the funnel, taking care not to disturb the sand. Rinse graduate with distilled H_2O and add to sample.

5. Turn on the suction using only light to moderate pressure to draw the water through the funnel.

6. Occasionally wash down the sides of the funnel with distilled water or tap water.

7. Concentrate the sample to the desired level (usually 5 mls.) taking care to remove the rubber tubing at the upper junction of the U-tube at the proper time.

8. Take the funnel from its rack and holding it over a small beaker, remove the stopper so that the 5 mls. of concentrated sample, the sand and the bolting cloth fall into the beaker.

9. Rinse the funnel with an additional 20 mls. of distilled water and catch it in the beaker.

10. One ml. of this concentrate is used for counting in the Sedgwick-Rafter cell.

Calculation

If a 1,000 ml. sample is reduced to 5ml. + 20ml. of distilled wash water, then the concentration factor is:

$$\frac{1,000}{25} = 40$$

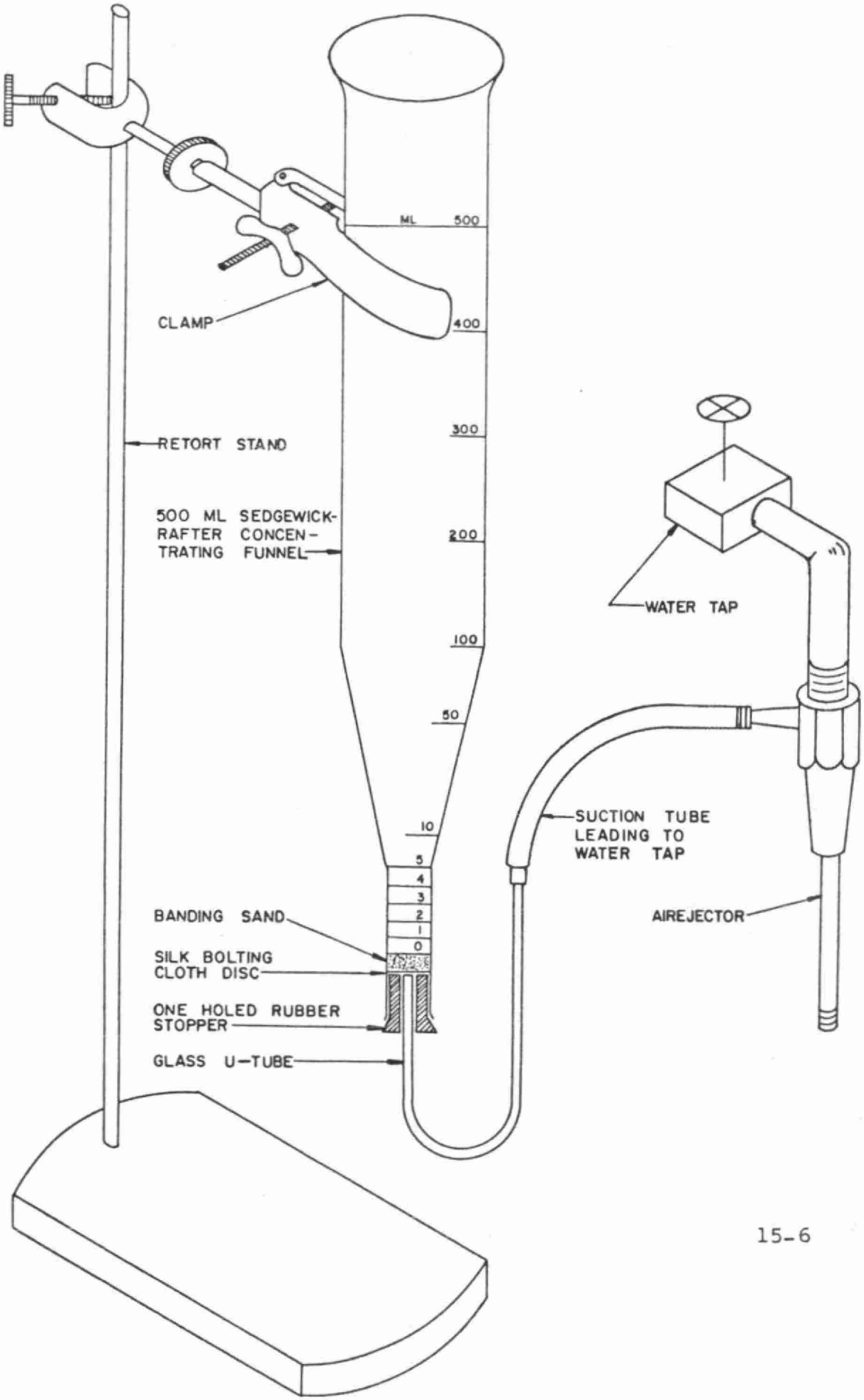
One ml. of the concentrated sample is placed in a Sedgwick-Rafter cell for enumeration. Five to twenty-five fields or up to two strips may be counted depending on the density of the organisms present. If counting by fields, it is considered that a degree of concentration that will provide no less than 10-20 organisms per field is essential to ensure reasonable statistical accuracy.

In determining the total count, the following formula is used:

$$\frac{\text{total square microns X factor (varies with fields counted)}}{\text{400 X concentration factor}}$$

The concentration technique should not be used if the sample contains high levels of algae (especially filamentous forms which tend to clump) or large quantities of organic debris.

EQUIPMENT FOR CONCENTRATING ALGAE



NOTES

CALIBRATION OF PLANKTON COUNTING EQUIPMENT

A Whipple Plankton Counting grid must be used in microscopes to delineate the width of counting strips and for measuring areal value of individual organisms. Since the optics of no two microscopes are exactly the same, it is necessary to "calibrate" each instrument against a known scale to determine the linear values of the lines or areal values of the squares making up the counting grid. The Whipple eyepiece must be calibrated for each magnification that is to be used. Microscopes having an adjustable tube length can be set so that the square which is part of the grid covers an area of one square mm. on the counting cell. This occurs when the tube length is 160 mm. However, most of the newer models of microscopes do not have an adjustable tube length.

A. Procedures Involved in Calibration

(1) Installation

To install the ocular micrometer in the eyepiece, carefully unscrew the upper lens and insert the disc, allowing it to slide down until it comes to rest on the shelf inside. Replace the lens and look into it. If the markings on the counting grid are not in sharp focus, remove the disc and turn it over.

(2) Using the Stage Micrometer

The stage micrometer is actually a tiny ruler which is placed on the stage to measure the dimensions of the lines making up the counting grid. The lengths of the various segments of the lines making up the grid should be determined and recorded on the Calibration Data sheet which is present in this section. This must be repeated for each combination of lenses employed.

(3) Lenses Normally Used in Counting Plankton

The 10X eyepiece and the 20X objective are normally used in counting plankton, to provide a total magnification of 100X. The 40X and 100X objective cannot be used with the S-R cell because of the short working distance beneath these medium power objectives.

B. Calculating Factors to Convert to Areal Counts Per Ml.

(1) Strip Counts

The S-R cell is 50 mm. long X 20 mm. wide by 1 mm. deep. The total volume of the cell is therefore 1000 mm^3 or 1 ml.

Factor for one strip - strip delineated by vertical lines in the counting grid. One strip the length of the cell in which the distance between the ends of the vertical lines making up the counting grid has been determined to be .5 mm. (or 500 microns) would have the following volume:

$$\text{Volume} = \text{length X width X depth}$$

$$= 50 \quad X \quad .5 \quad X \quad 1$$

$$= 25 \text{ mm.}^3$$

It is necessary to multiply the total count for each genus observed in one strip by a factor obtained by dividing the volume of the strip into the total volume of the cell.

Using our example, the Factor for 1 strip = $\frac{1000 \text{ mm.}^3}{25 \text{ mm.}^3} = 40$

If two strips are counted, then of course the multiplier factor would be cut in half - i.e. 20.

(2) Field Counts

If high numbers of algae are present in a sample it might not be necessary to count one full strip, although this is likely to be the exception rather than the rule.

When high numbers are encountered, ten fields may be examined and each field is delimited by the four lines making up a true square in the ocular grid. The basic relationship previously described still applies, as follows:

$$\text{Factor} - \frac{\text{volume of cell in mm.}^3}{\text{volume examined in mm.}^3}$$

The total volume of the area examined using 10 fields is determined by multiplying the total area of the 10 fields by the depth. Therefore, when the sides of the true square (i.e. one field) in the ocular grid represent a length of .3 mm. (or 300 microns) the volume for 10 fields is as follows:

$$\text{Volume} = (\text{side of Whipple field})^2 \text{ x depth (1 mm.) X (no. of fields counted)}$$

$$= .3 \text{ X } .3 \text{ X } 1 \text{ X } 10$$

$$= .9 \text{ mm.}^3$$

Therefore the Factor for 10 fields = $\frac{\text{Volume of cell in mm.}^3}{\text{Volume examined in mm.}^3}$

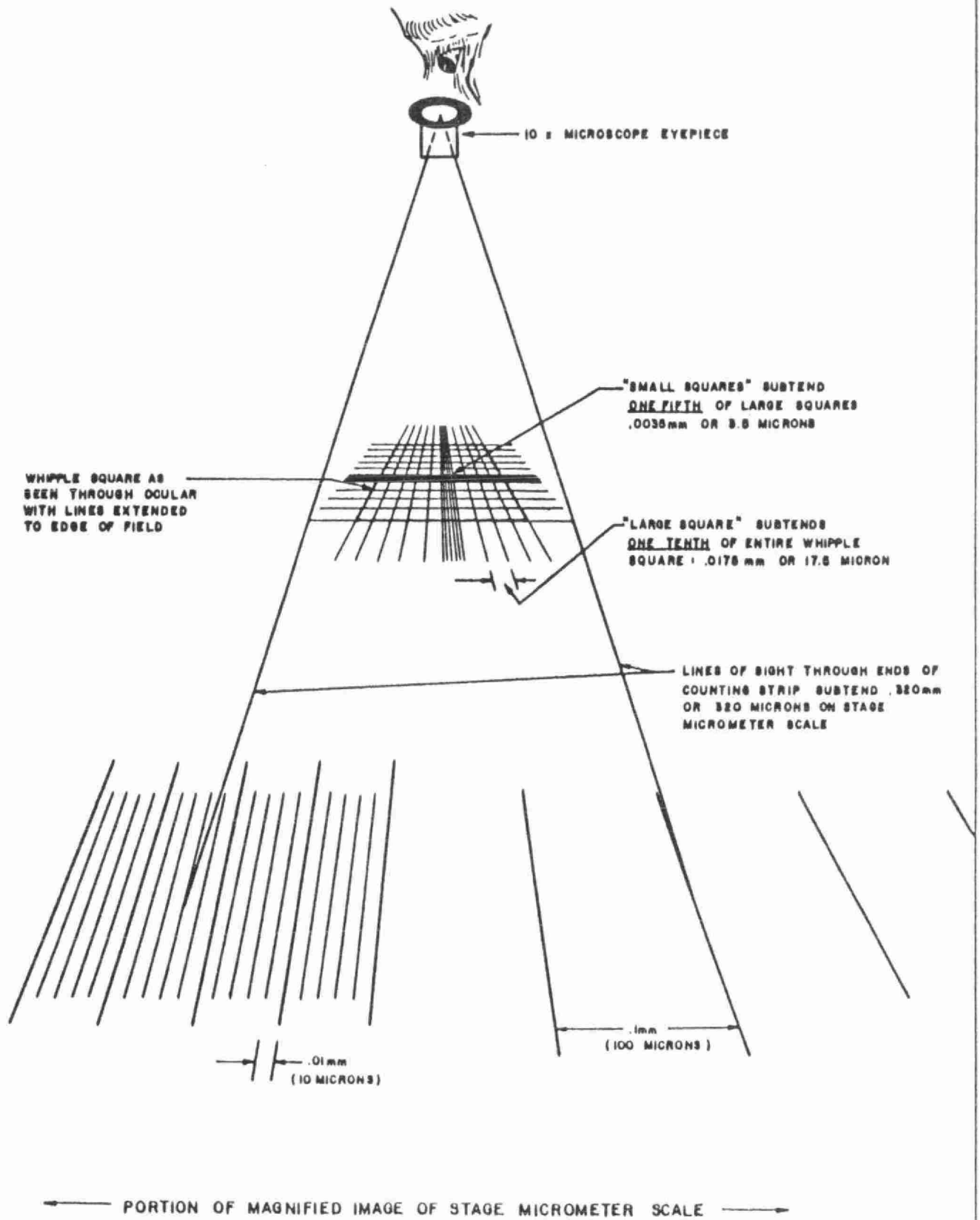
$$= \frac{1000 \text{ mm.}^3}{.9 \text{ mm.}^3}$$

$$= 1111$$

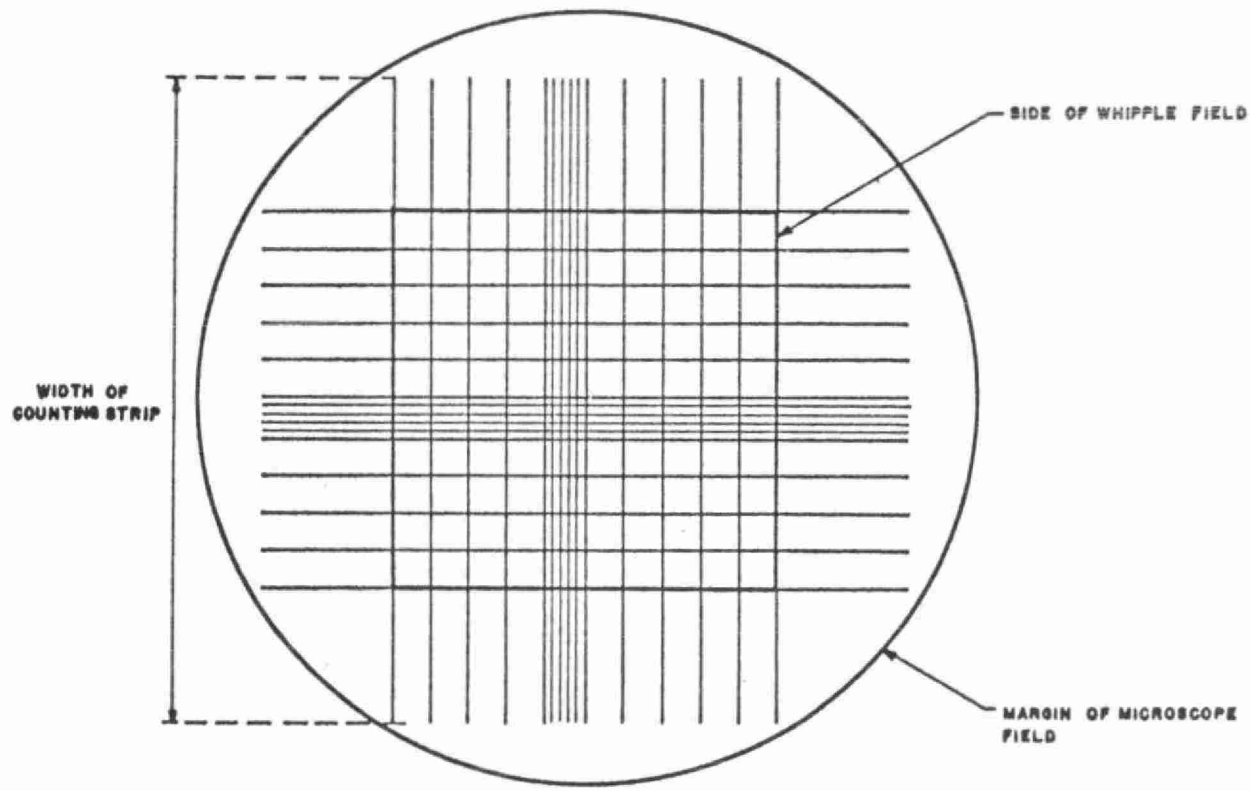
If 20 fields were counted, the factor would then become 555.

DIAGRAMATIC CALIBRATION OF WHIPPLE SQUARE FOR OWRC MICROSCOPE No. 1130

AS SEEN WITH 10x OCULAR AND 40x OBJECTIVE
(APPROXIMATLY 400x TOTAL MAGNIFICATION)



WHIPPLE COUNTING GRID
AS SEEN THROUGH MICROSCOPE EYEPIECE



MICROSCOPE CALIBRATION DATA

* 1MM = 1000 MICRONS

_____ Microscope No. _____

		Linear Dimensions of Whipple Square in Millemeters *			Width of Entire Field	Factor for Conversion to Count/ML.
	OBJECTIVE	WHOLE	LARGE	SMALL		(1 S-R STRIP)
Ocular						
Ocular						

NOTES

LIMNOLOGICAL ASPECTS OF WATER SUPPLY

INTRODUCTION

To date, the Algae Identification and Enumeration Course has included an introduction to the algae, the problems they create in water supplies and some consideration of the methods by which they may be controlled.

Problems created by the presence of algae in raw water supplies include:

(1) The reduction of filter runs, caused by the coating on the surface of the filters with large numbers of these minute plants, is probably the most common and serious problem that algae create for the waterworks operator.

(2) Algae are capable of producing tastes and odours that will persist through treatment and cause consumer complaints. Specific species in sufficient numbers cause particular odours (e.g. cucumber, grassy, fishy).

(3) In reservoirs, algae may grow attached to the walls where they form a heavy mass of material. This algal mass may be alive with crustaceans (e.g. Daphnia) and insect larvae. Occasionally, one of these little animals will come through a water tap and shake the confidence of the consumer in the purity of the supply.

Certain remedial measures which may be adopted include the standard practices of sedimentation, flocculation and pre-chlorination, as well as the installation of micro-strainers and the use of algicides such as copper sulphate or chlorine in holding basins. Taste and odours may be controlled by either superchlorination, break-point chlorination or where facilities permit, the feeding of activated carbon.

The purpose of this lecture will be to consider the relevance of limnology as it applies to certain aspects of water supply. It should be recognized that numerous interrelated factors govern the production of algae and other aquatic plants. These include physical, chemical and biological factors as follows:

(i) physical: temperature, density of the water, diffusion currents, horizontal currents, turbulence, amount of solar radiation, turbidity, ice-cover, etc.

(ii) chemical: pH; quantity of plant nutrients; oxygen content; presence of trace elements, such as manganese and iron; quantity of non-nutrient ions, Na^+; the ratio of free CO_2, HCO_3^-, $CO_3^=$, etc.

(iii) biological: quantity and nature of enemy organisms, interspecific and intraspecific co-operation and competition, metabolic rate, life span, rate of reproduction, etc. (from Davis, 1954).

Of these, the more important environmental factors are sunlight, water clarity, nutrients and temperature, and these will be considered in greater detail.

BASIC CONCEPTS OF FRESH-WATER ECOLOGY

The understanding of algae as they affect the waterworks operator requires a knowledge of the role of algae in the aquatic environment. Surface waters such as streams, rivers and lakes support active ecosystems in which there is a cyclic interchange between living and the non-living materials. The producer, consumer and decomposer organisms are the three main components in the aquatic ecosystem. The functional relationships within the system are illustrated in Figure 1.

The producers which utilize light energy synthesize organic matter (carbohydrates, fat and proteins) from inorganic sources (carbon dioxide, water and salts of phosphorus, nitrogen, sulphur and potassium). In fresh-water, the producers are represented by the chlorophyll-bearing algae and vascular aquatic plants. The consumers, in contrast to the algae, cannot manufacture their own food products directly from raw materials. Small consumers, collectively called zooplankton, obtain their food by eating the phytoplankton. These organisms are in turn eaten by larger consumers (i.e. insects and fish). Waste products and dead consumers, together with decaying algae and aquatic vegetation accumulate to form detritus which is used as food by the decomposers (bacteria, fungi and scavenger animals). These organisms bring about the mineralization of the detritus, thus completing the cycle.

All lakes, including those entirely unaffected by man's activities, are transitory bodies of water and are slowly becoming more fertile through run-off from the land which causes physical sedimentation and a gradual increase in dissolved mineral content. The impact of increases in levels of nutrients, including carbon, phosphorus and nitrogen, as well as other mineral salts such as calcium and silica, varies with climatic conditions, the shape and size of the lake basin, thermal conditions in the lake and turbidity and colour (which affect light penetration and hence the depth of the phototrophic zone). These factors interplay with the chemical content of the water to regulate the production of numerous algal species as well as larger attached forms of algae and vascular aquatic vegetation.

Where artificial (man-made) enrichment through inputs of domestic and industrial wastes, agricultural run-off and seepage from septic tanks occurs, the enrichment process which is scientifically known as "eutrophication" is tremendously accelerated. During periods of warm, calm, sunny weather, algae multiply and accumulate in large masses sufficient to form "water-blooms". Such imbalances may affect the waterworks operator by modifying the ecological system to the extent that excessive algal growths may develop to impart undesirable tastes and odours to water supplies and interfere with sand-filtration in water treatment plants.

Thus, the increased production in a lake reflects the presence of high concentrations of essential plant nutrients in the aquatic environment, just as farm crops are increased by farmers who apply artificial fertilizers to their fields. However, a highly productive lake may be a decided asset depending upon the point of view of the user. For example, the excellent fishing in the Kawartha Lakes is related to the fertility of the water. The abundance of game fish, such as muskellunge, walleye and bass, relates to the availability of small fish as a food source, which in turn are dependent on aquatic insects, water fleas and other invertebrates, all of which owe their existence to the algae. Thus, plentiful algae and plentiful fish appear to be two sides of the same coin in the Kawarthas.

EFFECT OF LIGHT

Plants are photosynthetic. Utilizing radiant energy from the sun, algae can produce carbon dioxide and water, and then assimilate these carbohydrates together with the liberated ammonia and other essentials to produce other algal cells.

$$\text{Carbon Dioxide} + \text{Water} \xrightarrow[\text{and chlorophyll}]{\text{in the presence of sunlight}} \text{Starches \& sugars} + \text{Oxygen}$$

This is an extremely simplified formula indicating what takes place as plants manufacture their own food. A balanced condition is provided by the fact that animals breathe the oxygen produced by plants in order to metabolize their food and release energy for movement and other bodily activities, at the same time producing carbon dioxide which is essential to the plants.

Any factor which limits photosynthesis would therefore restrict other life processes of the algae. McCombie(1953) reported that turbidity affects phytoplankton production by reducing the quantity and quality of light which is available for photosynthesis. Schenk and Thompson (1965) reported that the high turbidity levels may have had a strong impact by limiting phytoplankton populations at the Toronto Island

Filtration Plant from 1923-1954. However, it should be cautioned that any attempt to correlate changes in phytoplankton levels with a single physical-environmental factor such as the seasonal variation of turbidity is unjustifiable. It should be emphasized that physical, chemical and biological environmental circumstances acting simultaneously, must be taken into consideration.

EFFECTS OF NUTRIENTS

Of the many chemical constituents which are essential for the production of phytoplankton, attached forms of algae and "higher" aquatic plants, it is generally agreed that nitrogen and phosphorus are of major importance, since they are often in limited supply. While many other trace elements (manganese, iron, cobalt, nickel, etc.) are necessary for plant growth, there is no deficiency of most of these in the aquatic environment. Some algal forms prefer nitrogen in the NH_3 (NH_4) form while others have a preference for the (NO_3^-) form. Additionally, water surfaces are saturated with N_2. This serves as a source for the nitrogen-fixing algal forms. During the growing season, the various nitrogen forms become somewhat depleted while maximum concentrations are attained in the winter season. Major sources of phosphorus in surface waters are synthetic detergents, industrial wastes, treated sewage and drainage from agricultural lands. Sawyer (1965) concluded in a study of Wisconsin lakes that .30 mg/litre inorganic nitrogen and 0.01 mg/litre soluble phosphorus at the onset of a growing season could produce algal blooms. Edmondson (1968) however, states "The rule of thumb approach may be useful in some situations but cannot be generalized indefinitely from one to another... For instance, Lake Washington in the winter of 1950 exceeded this value for several months, developing a maximum of 0.016, but the lake was not regarded as producing algal nuisances then. In the winter of 1957, the maximum concentration was only 0.005 at a time when the lake was noticeably deteriorating. For reasons not understood, the algal population remained relatively dense during that winter and retained the phosphorus; the total phosphorus present was 0.034 mg/litre. Further, it was not demonstrated in the Wisconsin situation that some substance closely correlated with phosphorus was not actually controlling production."

TEMPERATURE EFFECTS

In the late spring and early summer, lake water is warmed by the sun and if the water remains relatively quiet, a temperature gradient is established in which the temperature declines exponentially with depth (Figure II). Similarly, since warmer water is lighter than colder water, the density of the water will not be the same from the top to the bottom. As the surface-water temperature continues to rise and becomes

correspondingly higher, more and more thermal resistance is offered to a mixing by wind action of surface water with the lower heavier water, until a temperature difference of 9°C. or more separates the surface water from that of the underlying water. This situation is called thermal stratification.

The establishment of this stratification establishes three well defined layers of water: the epilimnion, in which the water is essentially uniform; the thermocline, in which there is a rapid drop in temperature per unit of depth, and the hypolimnion in which the temperature from its upper limit to the bottom is nearly uniform.

EFFECT OF THERMAL STRATIFICATION ON LAKES AND RESERVOIRS

The upper layer of a stratified small lake or reservoir is characterized by warm water, good light conditions, an abundance of available oxygen and relatively small quantities of reduced chemical elements (i.e. iron, manganese). Under these conditions, the algae multiply, occasionally to the extent that some may die through exhaustion of nutrients, others from parasites and yet others from the grazing activity of the zooplankton. The dead cells as well as some live cells (some algal forms have a higher specific gravity than that of the surrounding water) sink into the depths. In the hypolimnion, decay of the dead algae and organic matter occurs. As a result, the available oxygen supply is depleted until at the mud-water interface, all of the free oxygen may be used up. An important consequence of this microbial decay is that nutrients (i.e. phosphate-phosphorus, iron, silica, and nitrogen) are regenerated but are confined to the hypolimnion because of the barrier presented by the thermocline. Furthermore, the available nutrients cannot be utilized by algal forms because of the reduced light intensity which is characteristic of the lower regions. However, once the water is mixed either by strong winds or by the fall overturn, these substances are dispersed throughout the entire water mass. In lakes and reservoirs where stratification periods are extensive, it would,therefore, be undesirable to depend on intakes located near the surface or too close to the bottom. An intake located in the photic zone (i.e. the epilimnion) would draw water containing tremendous quantities of algae, while a supply pipe located in the hypolimnion would contain undesirable sulphides, ferrous iron, manganous manganese, ammonia and a variety of odour producing organic compounds. It would be more sensible, in some instances, to take water from a depth near the lowest part of the epilimnion or in the thermocline zone.

In shallow, fertile lakes with no stratification, consistently high algal populations may prevail. Lakes of this type are seldom suitable as sources of water supply.

EFFECT OF THE INTERNAL SEICHE

Another potential difficulty which influences the quality of the raw water entering an intake (especially in small lakes or reservoirs) is the matter of the internal seiche. The internal seiche is a swinging, see-saw motion of the thermocline which occurs after the wind has blown strongly in one direction. Figure III illustrates the effects of the wind on a stratified body of water. "E" represents the epilimnion, "T" the thermocline and "H" the hypolimnion. When the wind blows from the west to the east, the warm epilimnetic waters move toward the east side of the lake. There is a piling up of the warmer upper waters to the east side of the water mass and a corresponding compensatory shift to windward of the thermocline. The hypolimnion remains undisturbed. If the wind is strong, a breakdown of the stratified layers on the eastern side of the lake occurs. This results in a shift in both the thermocline and the hypolimnion to the western side of the lake. With a stop in the wind, the regular stratification layers are restored but the thermocline continues to swing back and forth with corresponding shifts of the water in the epilimnion and hypolimnion. These movements are illustrated in the last three pictures of Figure III.

Consider now the case where there is an intake location extending into the thermocline from either the eastern or the western shore. With a drop in wind velocity the type of water reaching the supply pipe will differ from time to time according to the regular periodical pattern established by the motion of the thermocline. In a highly productive body of water, an internal seiche could mean that for a period of time, water containing massive growths of algae in the epilimnion would enter the water treatment plant; later in the day, water from the hypolimnion containing the decay - remains of organisms, reduced metallic ions (i.e. iron and manganese), ammonia, undesirable organic compounds, sulphides or even possibly hydrogen sulphide would enter the intake.

In large lakes such as the St. Lawrence Great Lakes, interrupted periods of summer stratification may drastically affect the distribution of polluted water entering a lake. In most instances, the temperature of the in-flowing polluted water is higher than that of lake water and tends to remain at or near the surface of the lake. If the thermocline is present, the polluted water enters and mixes with the upper, warmer epilimnetic waters. If, however, the epilimnion has been displaced by colder upwellings of the hypolimnion, the warm polluted water remains almost as a film on the lake surface. Indeed, pools of polluted waters have been reported (Matheson, 1963) lying immediately over an intake while the deeper, colder hypolimnetic waters were observed entering the water supply intake below.

In the absence of a thermal barrier (during periods of wind stress in the summer months and throughout the major portions of the autumn, winter and spring seasons), the polluted inflowing water disperses throughout all depths of the receiving water. If, for example, the polluted waters were to contain high levels of ammonia, the water treatment plant operator would have to contend with high chlorine demands and possibly the odours of nitrogen trichloride.

CONCLUSIONS

1. Limnological factors should be carefully considered and evaluated when selecting lakes or reservoirs as sources of water supply.

2. Shallow, productive bodies of water are likely to support high algal populations, thus causing notable increases in turbidity as well as influencing palatability and interfering with treatment processes.

3. Careful selection of intake locations is necessary in deeper lakes and reservoirs to minimize chemical and biological problems associated with temperature stratification.

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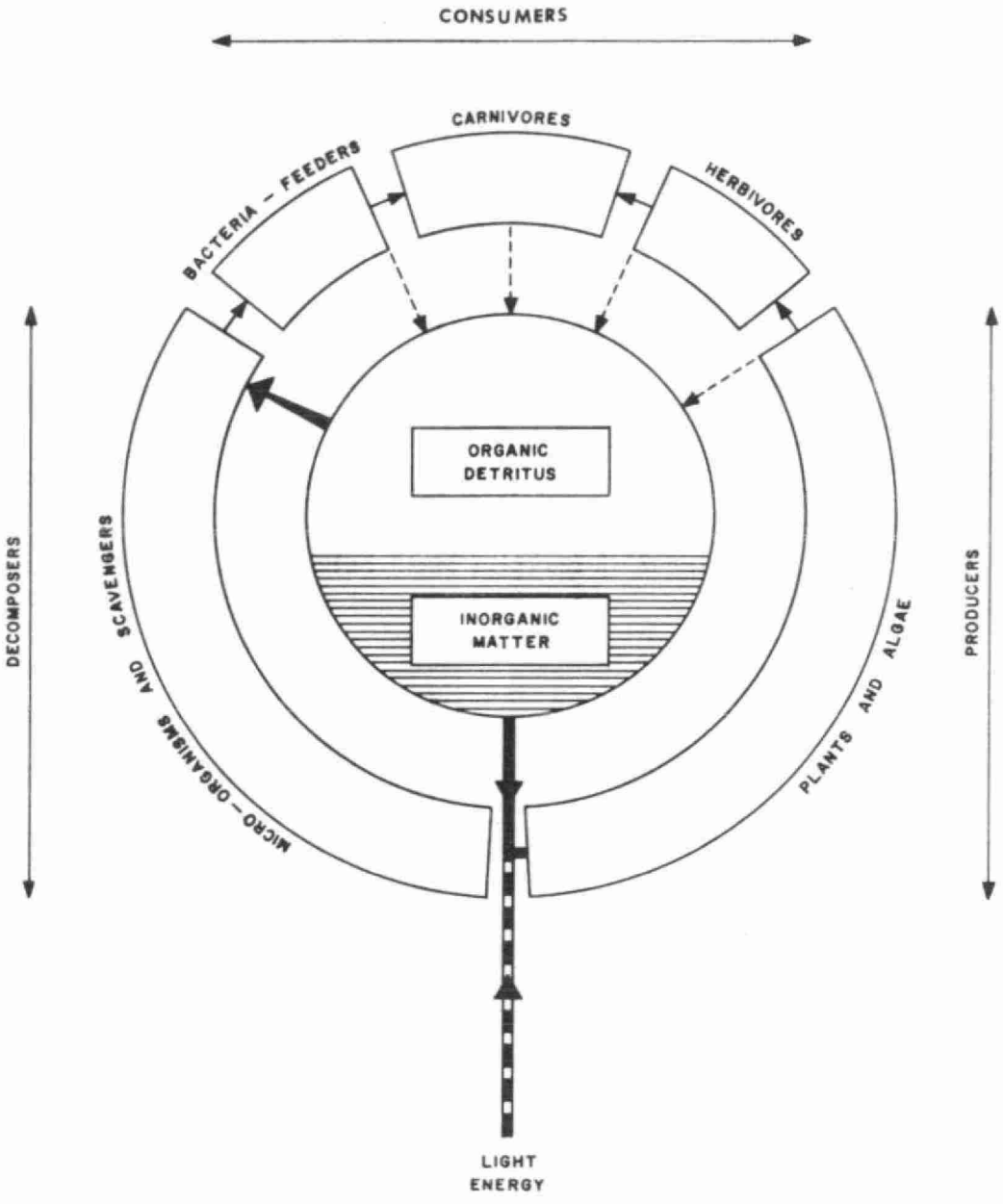
CONSUMERS
CARNIVORES
BACTERIA - FEEDERS
HERBIVORES
ORGANIC DETRITUS
INORGANIC MATTER
DECOMPOSERS
MICRO-ORGANISMS AND SCAVENGERS
PLANTS AND ALGAE
PRODUCERS
LIGHT ENERGY

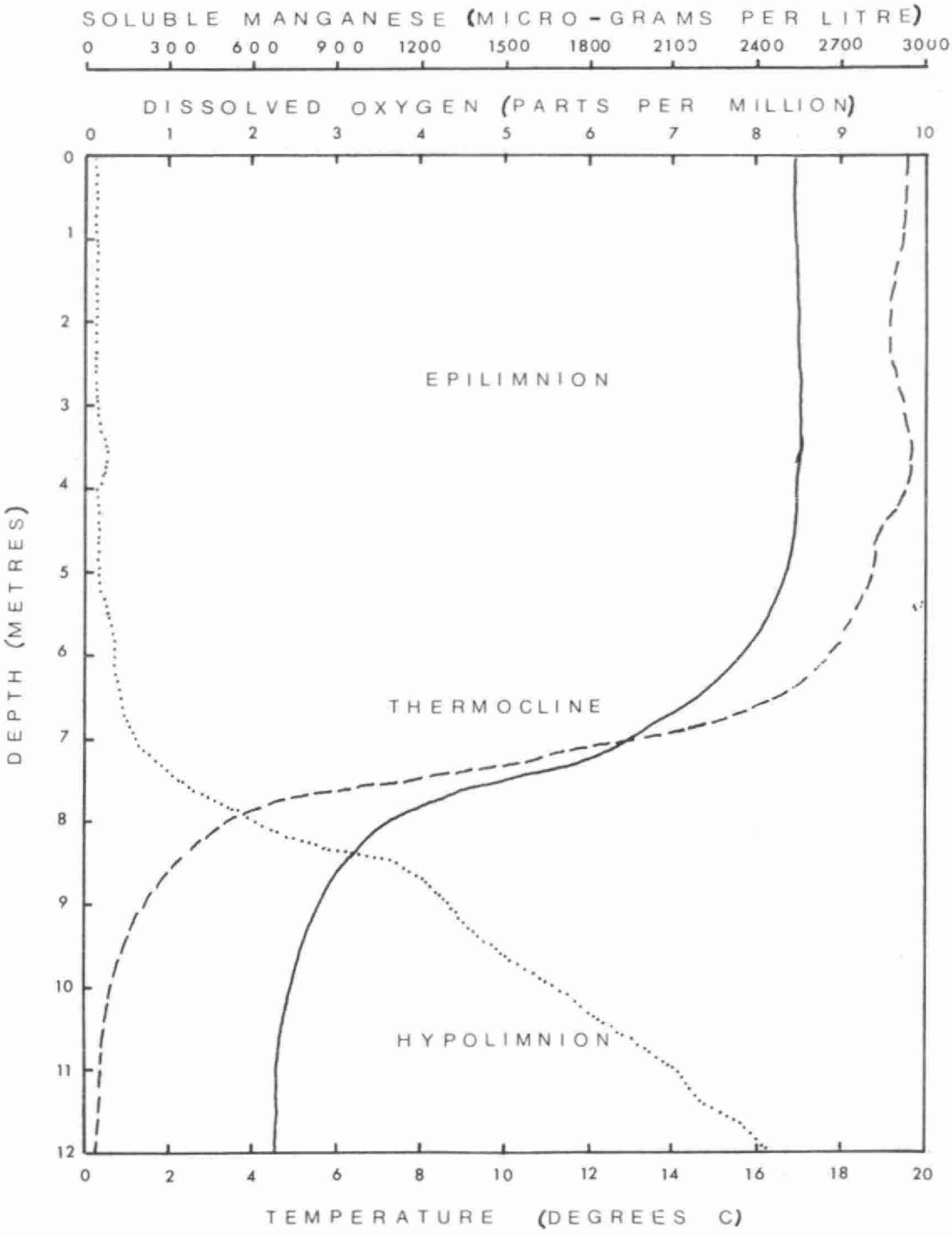

Figure 2. Mid-summer depth profiles of temperature (solid line), dissolved oxygen (broken line), and total manganese (dotted line) in a small lake or reservoir; from Lund 1967 and Welch 1951.

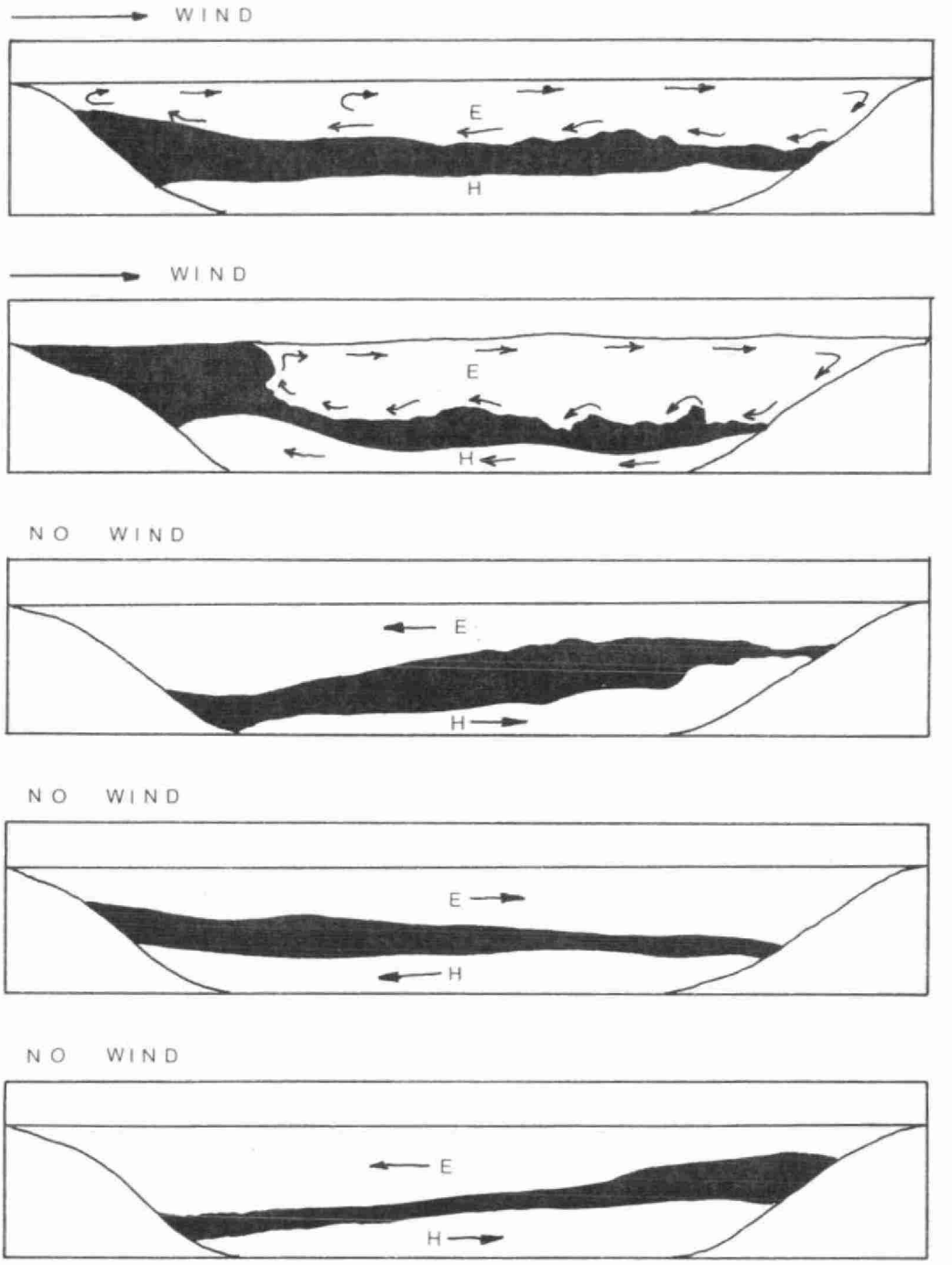

Fig. 3. Diagrammatic representation of an internal seiche. Flow of water is indicated by directions of arrows.

LABORATORY: QUALITATIVE EXAMINATION OF MIXED PLANKTON

1. Using the sample of raw water provided, study and identify the algae present and attempt to list them under the four major groups of algae which have been previously studied in detail. Use the texts available to help in your identifications and solicit the assistance of the instructors present.

2. If you would like additional practice with any one of the major groups ask the instructor to provide the necessary cultures.

3. Use the key to help sort the algae into proper groups. If you do not understand the terminology always check the glossary of terms.

LABORATORY: PLANKTON ENUMERATION - QUANTITATIVE

Using your own microscope, if you have one fitted with a suitable ocular micrometer, undertake a plankton count in one strip across the S-R cell, using the areal standard method. Record your count on a bench sheet.

Check with one of the instructors present to ensure that you are using the proper multiplier factor for your microscope.

Count a single strip using the sample which is available and which has previously been counted in our laboratory. Use a bench sheet and then record your count on a report form.

Continue practising counts using your own water sample or any of the other samples which are available. Compare results of your counts with our records.

Counting Procedures

Shake the sample thoroughly to evenly distribute the plankton in the sample, but not so hard that individual colonies will be disrupted.

Concentrate the sample in accordance with the instructions provided in a previous section(15.4).

Transfer 1 ml. of the sample to the S-R cell as described previously. Remove the aliquot from the sample bottle before the organisms have time to settle to the bottom. After the cover glass rotates properly into position, wipe off excess water lightly with a tissue. The cover glass should remain intact on the S-R even when turned upside down.

Set the S-R cell on the microscope stage and allow to stand for several minutes to enable the organisms to settle to the bottom of the counting cell.

Select ten randomly distributed fields for the count or one strip.

In a concentrated count, include all of the organisms lying within the Whipple field (see p. 16-4).

All of the organisms lying between the ends of the vertical lines in the ocular grid should be included in the count. Organisms which lie across the ends of these lines should be counted at the top, but not at the bottom if counting by strips.

Note: Examples of a "Bench Sheet" and a "Plankton Enumeration Sheet" are provided in 18-3 and 18-4.

For each field counted, identify and measure the individual cells and colonies as they are encountered by means of the lines and squares making up the ocular grid. The fine adjustment knob should be turned so that you can determine whether all of the organisms in each field are resting on the bottom. Any other organism observed at various levels must be included in the count. Determine the areal value for each cell or colony as they are observed and record on the bench sheet.

When your tally is completed for the ten fields or entire strip, multiply the total for each genus by the required multiplier factor to give you the total areal value for each in the entire ml. in the counting cell. Divide the total for each genus by 400 to obtain the number of areal standard units of each genus per ml. Always remember, when the sample has been concentrated, the total must be further divided by the appropriate concentration factor.

A 'survey count' should be made quickly using low power over ½ of the counting cell to determine the numbers of animal plankton present. These are recorded by number rather than by assigning areal values.

BENCH SHEET - PLANKTON ENUMERATION

Sampling Point (Raw Water etc.) raw water Date Sampled April 3, 1967 Sample No. 67-14

Date Analysed April7, 1967 by: G. J. Hopkins Factors: Algae 16.13

Concentration Factor 20

Enumeration Procedure Used: 2 Strips Zooplankton 0.80

Algae		Total Sq. Microns	A.S.U. per Milliliter (Total X Factor) 400 X conc. factor
Pediastrum	$55^d + 30^d + (35)^2(5)$	9,208	18.56
Chlamydomonas	$(8^d)32 + (10^d)8$ -or written as (10^d) 1111 111	2,238	4.51
Cyclotella	$20^d + 14^d + 26^d$	999	2.01
Fragilaria	(35X18) + (140X30)	4,830	9.74
Cryptomonas	12^d + (20x12) + 24^d + (18X10) + $(14^d)6 + 24^d$	2,151	4.34
Anacystis	$(20^d)2 + 40^d + 22^d + (35^2)3$ + (45X3) + 30^d	8,222	16.58
Melosira	(90X8) + (140X8) + (28X8) + (110X10) + (6400X105)	4,804	9.68
Oscillatoria	(50X2) + (105X2) + (90X2) + (210X2)	910	1.83
Scenedesmus	(24X10) + (18X12) + (30X10) + (24X10)	996	2.01
Synedra	(80X6)6 + (70X5)3 + (80X7) + (85X5)	4,385	8.84

Total Areal Standard Units Per Ml. 78.10

Zooplankton		Total Number	Total No. X Factor Concentration Factor
Protozoa	(unknown)6	6	5
Rotifer	Polyarthra 1	1	1
Ciliate	3	3	2

Total Number Zooplankton/ml. 8

PLANKTON ENUMERATION SHEET

Municipality Smith Falls Source Rideau River Sample No. 67-14
Sampling Point (Raw Water etc.) Raw Water Date Sampled April 3/67
Date Analysed April 7/67 By G.J. Hopkins
Enumeration Procedure Used 2 Strips
Volume Examined-Algae 31 cu. mm. Volume Examined-Zooplankton 31 cu. mm.
Enumeration Factor-Algae 16.13 Enumeration Factor-Zooplankton 16.13
Concentration Factor-Algae 20 Concentration Factor-Zooplankton 20

Algae	Total sq. Microns	A.S.U./ml.
Blue-greens		
Anacystis	8,222	17
Oscillatoria	910	2
Total		19
Greens		
Pediastrum	9,208	19
Scenedesmus	996	2
Total		21
Flagellates		
Chlamydomonas	2,238	5
Cryptomonas	2,151	4
Total		9
Diatoms		
Cyclotella	999	2
Fragilaria	4,830	10
Melosira	4,804	10
Synedra	4,385	9
Total		31
TOTAL AREAL STANDARD UNITS/ML.		80

Zooplankton		Total	No./ml.
Protozoa			
Ciliates	unknown	3	2
	unknown	6	5
Flagellates (unpigmented)			
Sarcodina			
	Total No./ml.		7
Microinvertebrates			
Rotifers Polyarthra		1	1
	Total No./ml.		1
Crustacea			
Cyclops			
Daphnia			
	Total No./ml.		
Miscellaneous			
	Total No./ml.		
Total No. Zooplankton/ml.			8

SPECIAL INFORMATION:
Colour Raw-15 Turbidity 4.5 ppm. Odour (if present) grassy
Tap-5 Temperature (Water) 36°F

REMARKS: Filter runs-24 hrs.; Cloudy to bright day; Winds-easterly, light;
Pre-chlorination dosage-1.14ppm.; Residual after 2 hrs.-0.40ppm.
Post-chlorination dosage-0.10ppm.; Residual after 2 hrs.-0.35ppm.
Feeding alum - 0.60ppm.
Note: See attached diagram for an unknown form found in this sample.

AREA OF CIRCLES IN MICRONS IF GIVEN THE DIAMETERS

Diameter 1-10		11-20		21-30		31-40		41-50		51-60		61-70		71-80		81-90		91-100	
1	1	11	95	21	346	31	755	41	1320	51	2043	61	2923	71	3959	81	5153	91	6504
2	3	12	113	22	380	32	804	42	1385	52	2124	62	3019	72	4072	82	5281	92	6648
3	7	13	133	23	416	33	855	43	1452	53	2206	63	3117	73	4185	83	5411	93	6793
4	13	14	154	24	452	34	908	44	1521	54	2290	64	3217	74	4301	84	5542	94	6940
5	20	15	177	25	491	35	962	45	1590	55	2376	65	3318	75	4418	85	5675	95	7088
6	28	16	201	26	531	36	1018	46	1662	56	2463	66	3421	76	4537	86	5809	96	7238
7	39	17	227	27	573	37	1075	47	1735	57	2552	67	3526	77	4657	87	5945	97	7390
8	50	18	255	28	616	38	1134	48	1810	58	2642	68	3632	78	4778	88	6082	98	7543
9	64	19	284	29	661	39	1195	49	1886	59	2734	69	3739	79	4902	89	6221	99	7698
10	79	20	314	30	707	40	1257	50	1964	60	2827	70	3849	80	5027	90	6362	100	7854
1-10		11-20		21-30		31-40		41-50		51-60		61-70		71-80		81-90		91-100	

FACTORS FOR OLYMPUS MICROSCOPES

Area Examined (read down)	Microscope Factor*(F)	Divide by 400	Concentration Factors (f) (read across top) 1	10	20	40
			Microscope Factor Divided by 400X Concentration Factor			
1/4 Strip	129.04	÷ 400 =	0.322	0.032	0.016	0.008
1/2 Strip	64.52		0.16	0.016	0.008	0.004
3/4 Strip	43.01		0.11	0.011	0.006	0.003
1 Strip	32.26		0.08	0.008	0.004	0.002
2 Strips	16.13		0.04	0.004	0.002	0.001
3 Strips	10.75		0.027	0.0027	0.00135	0.0007
4 Strips	8.065		0.02	0.0020	0.0010	0.0005
5 Strips	6.452		0.016	0.0016	0.0008	0.0004
1/2 cell	2.0		0.005	0.0005	0.00025	0.00013
1 field	8163.3		20.4	2.04	1.02	0.51
5 fields	1632.66		4.08	0.408	0.204	0.102
10 fields	816.33		2.04	0.204	0.102	0.051
15 fields	544.22		1.36	0.136	0.068	0.034
20 fields	408.165		1.02	0.102	0.051	0.0255
40 fields	204.08		0.51	0.051	0.0255	0.0128

* To convert area examined to area per ml.

Formula to convert total square microns to areal standard units per ml.

$$\frac{\text{Tot. Sq. Microns} \times (F)}{400 \times \text{Conc. } (f)}$$

LABORATORY: SPECIALIZED STUDIES RELATED TO ENUMERATION

Your microscope has been centred on one particular cell, colony or filament of algae. Assign what you believe to be the correct areal value to your organism and record the areal value and the number of the microscope. Return the organism to its original position when you finish. Go to all of the other microscopes in turn and repeat, recording the areal values and numbers of the microscopes as you proceed.

From the sample which has been provided, do an areal count involving ten fields and calculate the appropriate multiplier factor which you must use to project to areal standard units per ml.

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